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TITLE

The long-term effectiveness of efavirenz-based combination antiretroviral therapy, the impact of pharmacogenomics and pharmacokinetic interaction of artemisinin-based antimalarial therapy on efavirenz exposure among Ghanaian HIV-infected patients

Dr. Fred Stephen Sarfo

Doctor of Philosophy

University of Durham

School for Health

2013

Dedication

To Maame and Jason. Love always.

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Abbreviations

A- adenine

ACTG- AIDS Clinical Trialists Group

AIDS- Acquired Immunne deficiency Syndrome

ALT- Alanine transaminase

ARTS- artesunate

AST- Aspartate transaminase

AUC- Area Under the Curve

AZT- Zidovudine

BENCHMRK- Blocking Integrase in Treatment Experienced patients with a novel compound against HIV, Merck

BHIVA- British HIV Association

C- cytosine

CAR- Constitutive Androstane Receptor

cART- combination antiretroviral therapy

CDC – Centre for Disease Control

CKD-EPI- Chronic Kidney Disease- Epidemiological Collaboration

C_{max}- Maximum concentration

C_{min}- Minimum concentration

CYP – Cytochrome P450

CYP 2B6- cytochrome P450 isoform 2B6

CYP 2A6 – cytochrome P450 isoform 2A6

DART- Development of Antiretroviral therapy in Africa

D4T- stavudine

DHA- Dihydroartemisinin

DNA- deoxyribonucleic acid

DUET- Demonstrate Undetectable viral load in patients Experienced with ARV Therapy

EFV- Efavirenz

ELISA- Enzyme linked Immunosorbent assay

EMEA- European Medicines Agency

eGFR- estimated glomerular filtration rate

FDA- Food and drug administration

FOTO- Five-days on, two-days off

FSS- Fred Stephen Sarfo

G- guanine

HBV- hepatitis B virus

HBSAg- hepatitis B surface antigen

HCV- hepatitis C virus

HIV- Human Immunodeficiency Virus

HPLC- high performance liquid chromatography

HR- Hazards ratio

INCAS - Italy, the Netherlands, Canada and Australia Study

IRIS- Immune reconstitution inflammatory syndrome

IQR- Interquartile range

KATH- Komfo Anokye Teaching Hospital

LDL- low density lipoprotein

LTFU- Lost-to-follow up

MDRD- Modified Diet in Renal Disease

MERIT- Maraviroc versus Efavirenz Regimens as Initial Therapy

MOTIVATE- Maraviroc versus optimised therapy in viraemic antiretroviral treatment-experienced patients

MVC- Maraviroc

NA-ACCORD- North American AIDS Cohort Collaboration on Research and Design

NADE- Non-AIDS defining events

NFV- Nelfinavir

NRTI- Nucleoside (-tide) Reverse Transcriptase Inhibitor

NNRTI- Non-nucleoside reverse transcriptase inhibitor

NVP- nevirapine

OR- Odds ratio

PCR- polymerase chain reaction

PD- pharmacodynamics

PI- protease inhibitor

PK- pharmacokinetics

PMTCT- Prevention of Mother to Child Transmission

pVL- plasma viral load

PXR- pregnane X receptor

RAL- raltegravir

RNA- ribonucleic acid

ROP- Richard Odame Phillips

RXR - 9-cis retinoic acid receptor

SEM- standard error of mean

SMART- Strategies for Management of Antiretroviral Therapy

SNPs- Single Nucleotide polymorphisms

SSA- sub-Saharan Africa

START- Strategic Timing of Antiretroviral Treatment

T- thymidine

TB- tuberculosis

TDM- Therapeutic drug monitoring

TORO- T-20 versus Optimised Regimen Only

UGT – UDP-glucuronosyltransferase

ULN- upper limit of normal

WHO- World Health Organisation

3TC- lamivudine

Abstract

Dr. Fred Stephen Sarfo, The long-term effectiveness of efavirenz-based combination antiretroviral therapy, the impact of pharmacogenomics and pharmacokinetic interaction of artemisinin-based antimalarial therapy on efavirenz exposure among Ghanaian HIV-infected patients. PhD dissertation, Durham University, January 2013.

Introduction: In sub-Saharan Africa, HIV treatment is initiated with combination of antiretroviral medications comprising of a backbone of either stavudine or zidovudine plus lamivudine with a non-nucleoside reverse transcriptase inhibitor of either efavirenz or nevirapine. Efavirenz is highly efficacious, durable and well tolerated. The risk for toxicity of efavirenz is determined by several factors including single nucleotide polymorphisms in the hepatic enzymes responsible for its metabolism and concurrently administered medications such as antimalarials, which share common metabolic pathways. The aims of this dissertation are to assess the long-term effectiveness of efavirenz-based antiretroviral therapy and the impact of pharmogenomics and pharmacokinetic interactions of artemisinin-based antimalarial therapy on efavirenz exposure among Ghanaian HIV-infected patients.

Methods: The effectiveness of efavirenz- compared with nevirapine-based antiretroviral therapy was assessed retrospectively in nearly 4000 patients starting treatment between 2004 and 2010. The main outcome measure was a composite of toxicity, disease progression and attrition, and CD4 count changes. A prospective pharmacokinetic study of artesunate and efavirenz was conducted among 22 HIV-infected and 21 controls. Plasma efavirenz and artesunate/ dihydroartemisinin concentrations were measured using validated and standardised methods. Genotyping for single nucleotide polymorphisms in CYP2B6 G516T, T983C; CYP2A6*9B, UGT2B7*735 and *802 as well as CAR rs2307424 were performed for 800 patients with real-time polymerase chain reaction with allelic discrimination.

Results: Antiretroviral therapy was associated with robust CD4 increases. Efavirenz was comparable with nevirapine in composite outcomes but better tolerated. Artesunate was well tolerated when administered to HIV-infected patients on efavirenz. Single nucleotide polymorphisms in the CYP2B6 G516T and T983C were associated with increased plasma efavirenz concentrations.

Conclusions/Recommendation: Among this Ghanaian cohort, both efavirenz and nevirapine-based antiretroviral therapy were effective. The better tolerability of efavirenz compared with nevirapine means it can be safely used as the preferred first line non-nucleoside reverse transcriptase inhibitor in sub-Saharan Africa.

Contents

Table of tables

Table of Figures

Table of appendixes

CHAPTER ONE

Rationale of this dissertation and literature review

1.1 Rationale for this study

1.1.1 Research questions

The research questions that are answered in this dissertation are as follows:

1. How effective is efavirenz compared with nevirapine as the base of a dual NRTI cART among Ghanaian HIV-infected patients over the long-term?

2. What are the frequencies of selected single nucleotide polymorphisms of cytochrome P450 2B6, 2A6, UGT2B7 and constitutive androstane receptor (CAR) genes which have been predicted to impact on plasma efavirenz exposure? Are there any associations between pharmacogenomics of efavirenz and pharmacodynamics such as risk of toxicity and treatment failure among Ghanaian HIV-infected patients?

3. Is it safe to co-administer artesunate for treatment of malaria in patients on efavirenz without compromising the effectiveness and kinetics of either medications?

1.1.2 Why is this study important?

Nearly 10% of the world population reside in Sub-Saharan Africa but this continent is at the epicentre of the HIV/AIDS epidemic with more than 65% of all people living with HIV resident here[1]. The continent therefore has the highest rates of HIV-related mortality due predominantly to opportunistic infections particularly from tuberculosis. The overall impact of the HIV epidemic has been the reduction of life-expectancy by approximately 15 years in some regions in SSA[2]. This prompted a global response in 2001 with a commitment to increase access to life-saving antiretrovirals across the continent. This unprecedented roll-out of cART across SSA since 2002, has began showing beneficial results with reports of decreasing incidence of HIV-related mortality and morbidity within the first few months of therapy[1]. These benefits have been achieved with a very limited repertoire of first line cART comprising until recently of a dual NRTI backbone of either zidovudine plus lamivudine or stavudine plus lamivudine with an NNRTI-base of either efavirenz or nevirapine. Thus the advent of cART has made a once fatal disease into one of a chronic infection requiring life-long treatment with antiretrovirals. But only 37% of eligible patients were on cART in the African sub-continent by end of 2009, hence there is an urgent need to accelerate access to effective and well tolerated life-saving medications in the coming years[1]. As many more patients stay on these antiretrovirals, it has also become important to determine how effective NNRTI-based cART among HIV-infected patients over the long-term is.

Efavirenz has not yet been surmounted in its efficacy and safety across several studies as a third component of the triple cART compared with other classes of antiretrovirals[3]. In one large randomised controlled trial, nevirapine has been found to be non-inferior to efavirenz[4]. However, EFV has the advantage of superior outcome data to NVP in a number of observational studies[5-20], whilst also being less likely to cause serious adverse events such as rashes[4,21-23] or hepatotoxicity[24-28]. Furthermore EFV (unlike NVP) can be used with rifampicin in patients with TB co-infection. However, there remains concern regarding the teratogenicity of EFV in pregnant women, despite the lack of clear evidence of excess risk in pregnancy risk registers, such that it is still not recommended in women of child-bearing age who are not using effective contraception[29,30]. None-the-less a WHO-led survey has shown that around of two thirds of developing countries use NVP as the main first-line NNRTI in preference to EFV, largely due to lower cost and its availability as a generic fixed dose combination tablet.

A number of randomised controlled trials, one metanalysis and observational studies[5-20,31] have compared EFV and NVP as first-line therapies. There was little evidence favouring either in terms of virological outcomes, however more observational studies conducted in developing countries favoured EFV in terms of virological outcomes[7,8,14,15]. Few studies comparing these NNRTIs have been conducted in sub-Saharan Africa, and these have followed up patients for relatively short periods of time. Moreover few comparisons of NNRTIs have been conducted in settings with patients presenting with late HIV infection and starting ART at low CD4 counts, as is often the case in Africa. In Ghana, first-line ART consists of two nucleoside reverse transcriptase inhibitors (NRTI) and either EFV or NVP, if female with reproductive potential. Like many developing countries there is a high rate of tuberculosis and 17% of the HIV-infected population is co-infected with hepatitis B virus (HBV)[32], leading to further challenges with NNRTI-based ART in terms of drug toxicities and interactions. One aim of this study was to determine the long-term effectiveness and tolerability of first line ART, particularly comparing NVP and EFV, in a large cohort of patients attending a government HIV clinic in central Ghana. This was an ‘effectiveness’ not an ‘efficacy’ analysis because by definition efficacy relates to the extent to which a drug has the ability to bring about its intended effect under ideal circumstances, such as in a randomised clinical trial whereas effectiveness deals with the extent to which a drug achieves its intended effect in the usual clinical setting.

It is well known that the risk for toxicity to some ART is influenced significantly by the level of plasma exposure to the components of antiretrovirals. Specifically efavirenz has been shown to demonstrate a well-defined pharmacokinetic-pharmacodynamic relationship with supra-therapeutic exposure predisposing to higher risk of toxicity[33,34]. Single nucleotide polymorphisms (SNPs) in the enzymes responsible for the hepatic metabolism of efavirenz or nevirapine particularly the cytochrome P450 2B6 (CYP2B6) play major contributory roles in determining systemic exposure. A number of mutations, particularly the G516T and T983C in CYP2B6, have been found to be relatively common in Ghanaians[35,36], with the latter mutation having a gene frequency of around 7.3%. It is has also been postulated that in settings where polymorphisms in the CYP2B6 are common, the presence of other loss-of-function SNPs in the alternative oxidative metabolic pathway of efavirenz, namely the CYP2A6 enzymes, become important in predicting supra-therapeutic exposure. Given that supra-therapeutic exposure to efavirenz predicts risk of CNS toxicity with the potential for poor adherence, it is important to determine the prevalence of SNPs in the CYP2B6 and CYP2A6 enzymes involved in the oxidative metabolism of efavirenz as well as CAR and UGT2B7 involved induction of CYP2B6 expression and glucuronidation of oxidized efavirenz metabolites respectively and to assess their impact on efavirenz exposure in a Ghanaian HIV-infected population.

Other concurrently administered medications may also significantly influence exposure; for instance when treating patients with anti-tuberculous medications concurrently with cART, the induction of hepatic enzymes by rifampicin has been shown to lead to sub-therapeutic exposure to nevirapine hence increasing the risk of treatment failure, thus efavirenz is indicated for HIV-patients receiving TB treatment along with cART. Also the commonly used artemisinin-based antimalarial shares the CYP2B6 utilized in the metabolism of the NNRTIs and thus it has been predicted that potential pharmacokinetic and pharmacodynamic interactions between artemisinins and efavirenz may be of clinical interest in the African context. Thus it is important to conduct pharmacogenomic and pharmacokinetic studies to explore these questions especially as it is foreseen that efavirenz may become cheaper and more widely available within SSA in the coming years. This is within the strategic framework of the WHO towards providing effective anti-retrovirals that are minimally toxic for the treatment of HIV in developing countries[1].

1.1.3. Motivation for the study

The author has worked at the Department of Internal Medicine of the Komfo Anokye Teaching Hospital in Kumasi, Ghana for the past 9 years as a house-officer, a medical resident and as a specialist physician. When I started my training as a house-officer in 2004 the HIV clinic had just been commissioned and although I was not working in that clinic, I was involved in the management of several patients with advanced HIV disease referred from the HIV clinic for emergency admissions to the Medical block with various AIDS defining illnesses and ART-related toxicities. The most dramatic experiences in my mind were the severe and life-threatening skin rash from the NNRTIs. Unfortunately, a significant proportion of these patients with AIDS who came for admission died from various etiologies, predominantly from opportunistic infections due to late presentation at the clinic. An interest in HIV medicine was sparked by these experiences and in 2005, I had the opportunity to work at the clinic as resident in training. Over the years my encounter with patients on cART has shown that although most patients improved clinically and immunologically on treatment, different patients responded differently to treatment especially in the predisposition for developing adverse side effects and treatment failure. The extent to which the NNRTI chosen influences these outcomes is what has motivated this study.

To answer these questions, the author over the past 2 years had to collect retrospective data on ARV treatment outcomes in a busy outpatient clinic in Kumasi, Ghana where anti-retroviral therapy has been administered over the past 9 years at the time of writing this brief, but data presented in this dissertation involved follow up to 7.5 years. Data collected focused on clinical and immunological outcomes as well as toxicity of cART. Clinical outcome measures collected included deaths, loss-to-follow up, new AIDS defining conditions indicative of disease progression, non-AIDS defining events (NADEs) and infectious diseases neither fulfilling the criteria of AIDS-defining nor NADEs which nonetheless contributed to significant morbidity such as malaria. Immunological outcomes were assessed using the changes in the CD4+ T-cell counts over the course of treatment and risk for immunological failure as defined using the WHO criteria. Because patients are not routinely monitored with viral loads, virological outcomes were not available.

The risk for developing toxicity from efavirenz has been shown to be predicted by steady plasma concentrations which in turn is determined by its clearance predominantly via hepatic metabolism. To examine the impact of genetic polymorphisms in the metabolic pathways of efavirenz among Ghanaians and thus its systemic exposure, samples from nearly 800 patients were genotyped using real-time PCR with allelic discrimination for selected SNPs in CYP2B6, CYP2A6, UGT2B7 and

CAR and plasma efavirenz concentrations measured to explore the predictive associations between these SNPs and efavirenz exposure in the Ghanaian population of HIV patients. This was followed by a retrospective analysis of risk for efavirenz-associated toxicity and clinical outcomes for a subset of patients with complete data to evaluate the relationships between pharmacogenomic, pharmacokinetic and pharmacodynamic parameters.

This study was conducted with the future of ART in Sub-Saharan Africa in mind. If efavirenz should become cheaper and available as a fixed dose combination ART, would it be safe to administer to the vast majority of African patients who frequently harbour genetic polymorphisms which could potentially exposed them to supra-therapeutic levels of this potent NNRTI? Secondly, at its current cost compared with nevirapine, would there be an extra benefit of using a predominantly efavirenz-based first line over nevirapine in terms of effectiveness as governments, policy makers and donor agencies spread scant resources to increase access to life-saving antiretrovirals across Sub-Saharan Africa? Thirdly, can we safely use artemisinin-based anti-malarial combination therapy in patients on efavirenz?

1.1.4. The structure of the thesis

Data on long-term effectiveness of first line cART among four thousand and thirty-nine (4,039) Ghanaian HIV patients who initiated treatment between 2004 to 2010, together with pharmacogenomic data of eight hundred (800) patients of whom five hundred and thirty-one (531) were on efavirenz and twenty two (22) HIV-infected patients on efavirenz-based cART who were treated for malaria with artesunate to study the safety and pharmacokinetics of both anti-malarial and efavirenz are presented in this dissertation. Thus scope of this thesis spans from epidemiology through population pharmacogenomics and pharmacokinetics. The challenge was how to write a coherent piece that inculcated the multi-faceted and complicated management issues associated with long-term HIV treatment and to blend this with the pharmacology studies and to reduce the vast wealth of data into reasonably sized chapters.

In chapter one, the author states the rationale for this study and reviews relevant literature to capture themes on HIV epidemiology in Sub-Saharan Africa, factors influencing efficacy of antiretroviral therapy, the efficacy of efavirenz-based cART compared with other classes of antiretrovirals and concludes by reviewing the pharmacology of efavirenz by focussing on pharmacogenomics of efavirenz highlighting the cytochrome P450 2B6 as a highly polymorphic enzyme exhibiting profound inter-individual variability.

It became obvious during the write up of the methods sections of the main chapters of the thesis that, some statistical methods were reiterations (the effectiveness chapters) while others had labyrinthine methodology (pharmacogenomic chapter) which obstructed the flow of the story from the introduction to the results and discussion sections. This led to the creation of chapter 2 which is a compilation of methodology for chapters 3 through to chapter 8.

The main results of the body of work in this dissertation begins in chapter 3. Here the author presents an analytical description of the baseline characteristics of 4,039 patients at initiation of cART focusing on demographic, clinical and laboratory parameters of these patients and went in-depth to assess the risk factors and prevalence of HIV-related renal impairment as assessed by estimated glomerular filtration rates from serum creatinine measurements. This was followed by a longitudinal analysis of the clinical and immunological outcomes of first line cART emphasising the robust and sustained CD4 increases over the long-term from baseline values among patients who remained on treatment and also the decline in the incidence rates of deaths, loss-to-follow-up, AIDS-defining and NADEs over the course of time among patients who remained on treatment (chapter 4). Given the importance of toxicity on the durability of any cART, chapter 5 is presented to highlight the incidence rates and risk factors of five common ART-associated toxicity namely anaemia, skin rash, neuro-psychiatric toxicity, severe hepatic toxicity and mitochondrial toxicity. The preceding chapters (3 to 5) culminates in chapter 6 where the effectiveness of efavirenz-based cART is compared with nevirapine-based cART using a composite end-point of deaths, disease progression and all-cause discontinuation of therapy with sensitivity analysis included to evaluate the impact of the NRTI-backbone on these outcomes. The studies on the pharmacology of efavirenz among Ghanaian HIV-infected patients begins with a prospective pharmacokinetic study of artesunate monotherapy for treatment of clinically suspected malaria among HIV-infected patients on efavirenz with a control group of patients with symptoms of malaria whose HIV-status was not known (chapter 7). Chapter 8 focuses on the impact of single nucleotide polymorphisms in the CYP 2B6, CYP 2A6, UGT 2B7 and CAR on mid-dose exposure to efavirenz by assessing the frequency of these polymorphisms, the pharmacokinetics of efavirenz and the pharmacodynamic impact of these polymorphisms on the risk of developing CNS toxicity on efavirenz and also on the risk of immunological failure. To conclude, chapter 9 summarises the major findings, limitations and recommendations for further studies to improve our knowledge.

1.1.5. About the Author.

Dr. Fred Stephen Sarfo is a medical doctor at the Komfo Anokye Teaching Hospital where he has been practising since 2003. He completed his medical education at the School of Medical Sciences of the Kwame Nkrumah University of Science and Technology with BSc Human Biology in 2000 and MBChB degree in 2003. He did his house jobs at the Department of Medicine and Obstetrics and Gynaecology in 2004 and entered into residency training in 2005 with the West Africa College of Physicians. He successfully completed his membership and fellowship training certification examination in Internal Medicine with this college in April 2008 and October 2010 respectively. Over the past 7 years, Dr. Sarfo has been working at the HIV Clinic in Kumasi where he has been involved in patient management since early 2005. His major research interests have been on infectious diseases such as HIV, HIV-HBV co-infection, HIV-TB co-infections and has also been involved in pinioneering work on the immunopathogenesis and antimicrobial therapy for a neglected tropical disease called *Mycobacterium ulcerans* disease. Prior to this dissertation the research experience accrued by the author has ranged from laboratory methods in DNA and RNA extraction techniques for real-time PCRs and microarrays, ELISAs for proteomics and lipid extraction for mycobacterial lipids. The author therefore had a novel challenge of collecting data and performing statistical analysis from an epidemiological perspective with a large database to assess outcomes of effectiveness of cART. The work on pharmacology of efavirenz and artesunate was also a new experience for which the author is very grateful to staff at both the department of Pharmacology and Therapeutics of the University of Liverpool and the Liverpool School of Tropical Medicine. Most of this study was conducted in Kumasi, Ghana with short-term academic visits to the UK for analysis and meetings with supervisors to discuss progress with supervisors. The journey has been very exciting with lots of opportunities for learning. All these experiences have helped shape my thought processes and deepened my appreciation of statistical modelling, pharmacogenomics and pharmacokinetics as it pertains to patients whom I will encounter either in consulting rooms at the HIV clinics or on the wards and has better placed me to launch a career as research clinician.

1.2.0. REVIEW OF LITERATURE

1.2.1 Human Immunodeficiency Virus (HIV)

1.2.1.1 Epidemiology of HIV/AIDS

Acquired Immune Deficiency Syndrome (AIDS) has claimed the lives of more than 25 million people worldwide since the clinical syndrome was first described in 1981 (UNAIDS/WHO 2007). The Human Immunodeficiency Virus (HIV), initially referred to as either the Lymphadenopathy-associated Virus (LAV) or the Human T-cell Lymphotropic Virus type III (HTLV-III) was first identified as the causative agent of AIDS in 1983 [37,38]. The predominant route of transmission of HIV is through unprotected sexual intercourse (>75%), followed by vertical transmission from mother to baby, during pregnancy, at birth or through breast feeding (5-10%) and to a lesser extent via the parenteral route (<2%) (through injection drug use and/or injection/transfusion of contaminated blood products). Certain sexual activities carry a higher risk of infection than others; for example, unprotected anal sex carries a greater risk of infection than either vaginal [39, 40] or oral sex[41]. It is also apparent that the presence of other sexually transmitted diseases (STDs) significantly increases the risk of becoming infected with HIV.

HIV is a global pandemic with an estimated 33.3 million (31.4 million-35.3 million) persons living with the virus worldwide at the end of 2009 compared to 26.2 million in 1999 according to epidemiological data from UNAIDS/WHO[1]. In spite of intensive research efforts and the massive roll-out of treatment interventions, HIV/AIDS continues to pose the most serious infectious disease challenge to public health. This is particularly true in Sub-Saharan Africa where over 22.5 million (20.9 million-24.2

million) people are HIV infected (approximately two-thirds or 68% of the global HIV infected population). 61% and 90% of all HIV infections in women and children respectively occur in Sub-Saharan Africa. Indeed in SSA ~ 1.3 million (1.1 - 1.5 million) of the global total of 1.8 million (1.6 – 2.0 million) deaths in 2009 were attributable to HIV/AIDS accounting for 72% of all HIV/AIDS related mortality worldwide (UNAIDS/WHO 2010).

Within the Sub-Sahara African region the Eastern and Southern African countries are the most devastated with an average national prevalence ranging from 15 -35% of populations affected (Figure 1.1). However like the trend globally, the prevalence of HIV infection (percentage of persons infected with HIV) is stabilising, although the number of persons living with HIV continues to increase mainly due to an increasing number of newly acquired and diagnosed infections (albeit, at a reduced rate), longer survival times which is a corollary of the introduction of antiretroviral therapy (ART) and the unprecedented expansion of ART in Africa and other developing countries. For instance, the number of new infections in 2009 in Sub-Saharan Africa was 1.8 million compared with 2.2 million in 2001. An estimated 5.2 million people in low- and middle-income countries were receiving life-saving antiretroviral therapy by end of 2009 representing an increase of 1.2 million people or 30% over the number receiving such treatment 12 months earlier. In Sub-Saharan Africa nearly 37% (34-40%) of people eligible for treatment were accessing ARV by December 2009 and this unprecedented roll-out of ARV has translated into an estimated 320,000 (or 20%) fewer AIDS-related deaths in 2009 than in 2004. Clearly, the current data demonstrate that investments in the HIV response has started to yield fruitful dividends in reducing discrimination and stigmata, helping people access information and services to reduce their risk of HIV infection and delivering the treatment, care and support that will extend and improve the lives of people living with HIV.

Ghana, a West African developing country with a population of 24.5 million, is in the stable phase of the HIV pandemic with an estimated adult (aged 15-49) HIV national prevalence of 1.8% in 2009[1] from the estimated 3.1% estimated prevalence at the end of 2003. It is estimated that 260,000 (low estimate of 230,000; high estimate of 300,000) people of all ages in Ghana are living with HIV/AIDS and the age group predominantly affected are within their reproductive years. The highest prevalence is found in metropolitan cities and big towns where there is a lot of commercial activities. The Ghana AIDS Commission is the coordinating body for all HIV/AIDS-related activities in the country and through the National strategic programme has set targets for reducing new infections, addressing service delivery issues and individual and societal vulnerability, and promote the establishment of a multisectoral, multidisciplinary approach to HIV/AIDS programmes. Substantial funding for HIV/AIDS activities from multilateral and bilateral partnerships for activities such as the Global Fund to Fight AIDS, Tuberculosis and Malaria and the World Bank-funded treatment acceleration program for public-private partnership in HIV/AIDS management and several others. These initiatives have been rewarded by a downward trend in HIV prevalence observed in national surveys since 2006 and this has been attributed to the increased awareness of the disease and increased uptake of protected sexual practises public health messages. An estimated 30,265 patients were on antiretroviral medications representing 25% ART coverage at the end of 2009 in Ghana, an indication of a need to improve access to ART in the country

Figure 1.1. National HIV prevalence map of percent of men and women aged 15 to 49 years with HIV infection at 2010. Source: Blake Zachary ICF Macro at http://www.hivspatialdata.net

1.2.1.2 HIV Structure

HIV is a retrovirus, meaning its genetic information is stored in the form of ribonucleic acid (RNA) instead of deoxyribonucleic acid (DNA) and the virus therefore requires reverse transcription in order to replicate. HIV belongs to a sub-family of lentiviruses, which include the simian immunodeficiency virus (SIV) in monkeys, feline immunodeficiency virus (FIV) in cats and related viruses in sheep, goats and horses; these retroviruses are characterised by the lengthy time period between infection of the host and clinical manifestation of symptoms. Two strains of HIV have been identified (HIV-1 and HIV-2), the latter was identified 2 years later after HIV-1[42]. HIV-1 is derived from the SIV of chimpanzees called *Pan troglodyte troglodytes* while HIV-2 which is quite dissimilar is genetically closer to the sooty mangabey (called *Cercocebus atyps*) virus. HIV-2 is associated with a slower and more benign disease course, is more difficult to transmit via sexual and parenteral routes than HIV-1 and is endemic in West Africa. However HIV-1 is the major cause of AIDS worldwide and even in West Africa.

HIV-1 is further divided into three groups, group M (main group, >98%), group O (outlier, <1%), and group N (new, <1%). Group M is responsible for the majority of infections worldwide and is further subcategorised into recognised phylogenetic subtypes or clades namely clades A (23%), B (8%), C (56%), D (5%), E (5%) and subtypes F-K (3%). There are also recombinants, which contain a mix of these subtypes.

HIV-1 viral particles are spherical in shape and 100nm in diameter. A double layer of lipoprotein membrane, also known as the viral envelope, surrounds the virus. Integrated within the lipid membrane are 72 glycoprotein spikes, each composed of trimers of gp41, a transmembrane protein, and an external glycoprotein gp120. The HIV matrix proteins consisting of the p17 protein, lie anchored to the viral envelope and encompasses the viral core. The viral core or capsid, contains the viral protein p24 which surrounds two single strands of HIV-1 RNA and the enzymes required for viral replication, including reverse transcriptase p66, integrase p32 and protease p11. The HIV-1 RNA is a protein-nucleic acid complex, composed of the nucleoprotein p7 and the reverse transcriptase.

The viral genome consists of 9 genes. Structural proteins such as glycoprotein 160, which is subsequently cleaved to form the gp120 surface molecule and the gp41 transmembrane molecule is encoded by the *env* gene whiles the *gag* gene encodes the precursor to the capsid molecules p24, p17, p9 and p6; the latter enabling budding of the virus. Viral enzymes including reverse transcriptase, protease and integrase are encoded for by *pol*. There are also regulatory genes- *tat*, which regulates transcription, *rev*, which aids translation and *nef*, which down-regulates expression of CD4, major histocompatibility complex (MHC) class 1 proteins and interleukin-2, and recruits lymphocytes to infected macrophages to aid spread. Other accessory genes include *vif* and *vpr*. The strands of RNA are flanked by repeated sequences known as long terminal repeats (LTR), which do not encode any viral proteins but play a role in regulating gene expression[43].

1.2.1.3 HIV Replication Cycle

The CD4 surface receptor, a 58kDa monomeric glycoprotein, is the primary target for HIV entry into the host cell. CD4 is present in ~60% of T-cell helper lymphocytes, T-cell precursors, monocytes and macrophages, eosinophils, dendritic cells and microglial cells of the central nervous system (CNS). However, in addition to CD4, human co-receptors (CCR5 and CXCR4) also located on the host cell surface are necessary for viral entry. HIV-1 tropism refers to the cell type that HIV preferentially infects and replicates in, such that T-tropic (X4) HIV-1 isolates mainly infect activated CD4 T-cells using the CXCR4 co-receptor; M- tropic (R5) isolates are able to infect CD4 bearing macrophages, monocytes and T-cells; whereas the dual-tropic virus can use either CCR5 or CXCR4. During the early course of HIV-1 infection, M-tropic isolates predominate. In fact, M-tropic HIV-1 isolates are preferentially transmitted even if the donor predominantly harbours T-tropic isolates. It has been postulated that preferential transmission may be due to selective transportation of M-tropic isolates by sub-mucosally located dendritic cells or because local cytokine/ chemokine elaboration favours the replication of the M-tropic viruses. In contrast, the more virulent X4 or dually tropic viruses, which are probably able to infect a wider spectrum of cell types, evolve during the more advanced stages of infection.

The replication of HIV can be divided into 6 stages: attachment/ uncoating, reverse transcription, integration, transcription, translation and assembly/release; as schematically illustrated in Figure 1.2 and described below.

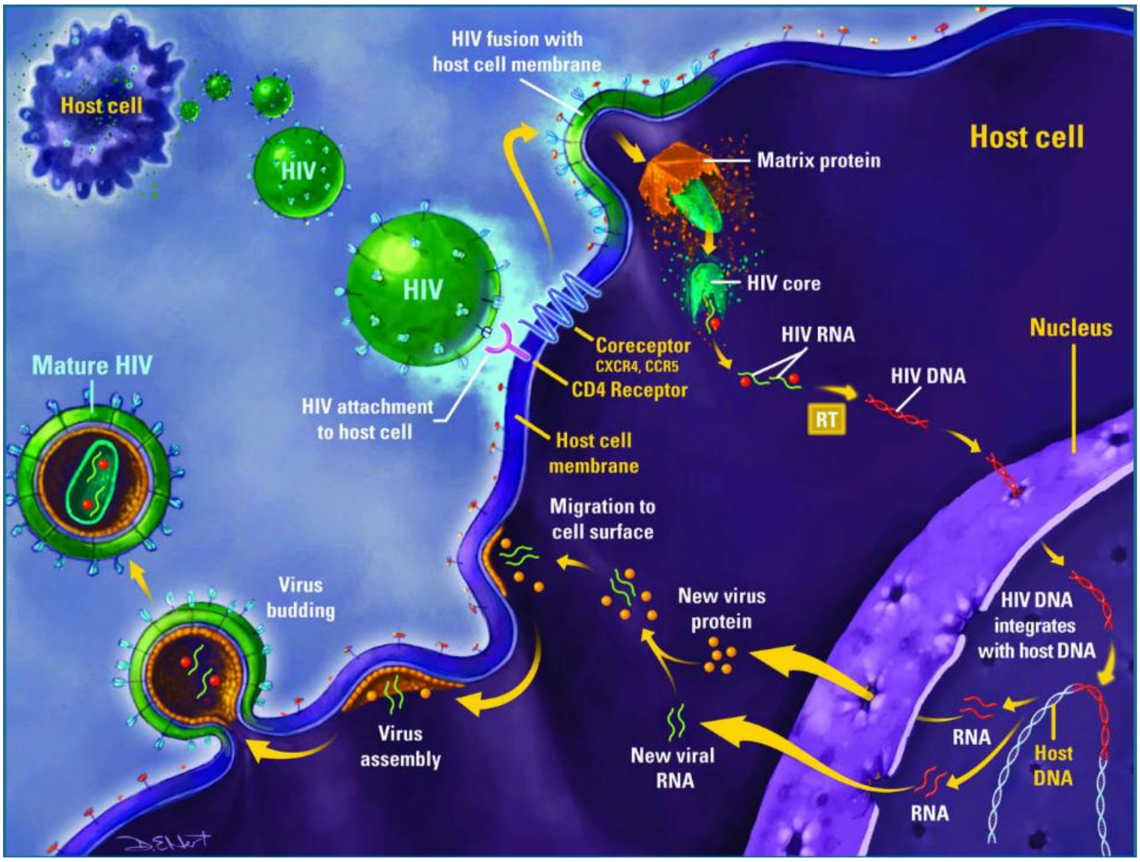


Figure 1.2. The life cycle of HIV-1.

Attachment/ uncoating: Binding of gp120 protein of the HIV to the CD4 surface receptor on the host cell induces a conformational change in gp 120, which facilitates a more efficient interaction with co-receptors CCR5 and/or CXCR4. Following binding to these co-receptors another conformational change in gp41 ensues enabling the fusion of the viral and host cell membrane. Subsequent to membrane fusion, the capsomere is uncoated and the contents of the viral particle are released into the host cell cytoplasm.

Reverse transcription: The genetic information stored in the RNA of HIV must be reverse transcribed into proviral HIV DNA in order for the virus to replicate. This process is mediated by the viral reverse transcriptase enzyme, which utilises host deoxynucleotide triphosphates (dNTPs) in the cytoplasm of the host target cell.

Integration: Once formed, proviral DNA is transported into the host cell nucleus where cellular activation causes integration of the proviral HIV DNA into the host cell genome, catalysed by the viral integrase enzyme. This newly incorporated viral DNA is known as a provirus. This provirus will remain inside the host cell awaiting stimuli from cellular transcription factors, such as NF-κB, to initiate transcription[44]. However without cellular stimuli the provirus may remain dormant within the target cell; for example, as proviral non-integrated HIV DNA in quiescent CD4 T-cells and in monocytes, macrophages and microglial cells and represent an important source of cellular reservoirs of HIV[45], which is one of the principal reasons why complete eradication of HIV is unattainable at the moment.

Transcription: following a cellular stimulus, transcription of host cell DNA occurs, which now includes the provirus. Transcription is mediated by host RNA polymerase and using endogenous dNTPs of the host.

Translation: the messenger RNA (mRNA) is transported out of the cell nucleus and translated into viral polyproteins using host amino acids and ribosomes forming large precursor molecules.

Assembly/release: new viral core proteins, enzymes and viral RNA migrate towards the cell surface where the large precursor viral proteins are cleaved by the HIV-1 protease, resulting in the formation of infectious viral particles, which bud through the host cell membrane into the extracellular space[46,47]. During the budding process, virus lipid membranes can incorporate various host cell proteins and become enriched with certain phospholipids and cholesterol[48,49].

1.2.1.4 Natural History of HIV Infection

The ' natural history' of HIV infection refers to disease progression in the absence of antiretroviral treatment and can be described in 3 phases namely primary or acute infection, latent or asymptomatic infection and symptomatic infection.

Primary infection is defined as the time period from initial infection with HIV to the development of an antibody response (seroconversion) and typically lasts between 1 to 3 months. It is characterised by a surge in plasma viremia due to unrestrained viral replication, with plasma viral loads (pVL) reaching over 100 million copies/ml in the absence of any detectable adaptive immune response[50,51]. Following seroconversion there is a marked decline in the number of CD4 T-lymphocytes as a result of both destruction by HIV and migration to peripheral lymph tissue[52,53]. During the period patients may develop a generalised rash, sore throat and lymph gland swellings, which is frequently misdiagnosed as flu by clinicians particularly because no HIV specific antibodies are detectable during this early phase of infection. Although higher pVL during primary infection are not directly indicative of disease progression, they are linked to the severity of these initial symptoms. Certainly, it has been observed that individuals presenting with more severe symptoms during acute infection tend to have poorer long-term clinical outcomes and progress more rapidly to AIDS[54-56].

An immune response capable of controlling viral replication develops over the following weeks, which is evidenced by a diminution of the high pVL. This coincides with a concurrent increase in the CD4 count, although levels rarely re-establish to pre-infection values in the absence of antiretroviral therapy. The high plasma viremia during the primary infection stage makes patients highly infectious hence accurate early diagnosis of acute HIV infection is important, as infection of sexual partners can be potentially prevented.

Latent infection refers to the time period in which equilibrium is established between rate of viral replication and the host immune response. This is often referred to as the viral 'set point' where the level of viral RNA remains constant at approximately 10^3-10^5 copies/ml[57, 58] and is a strong predictor of the speed of HIV disease progression following infection[59]. In the absence of antiretroviral treatment, this period of clinical 'latency' can last for 8-10 years or more[52, 60, 61], and during this period many infected individuals do not present with any clinical symptoms of the disease. However, the term latency is misleading, giving the high turnover of virus (up to 10^{10} new virions per day) and the relentless daily destruction of CD4 cells[62].

A number of host factors have been identified to influence the time spent in clinical 'latency', including gender[63], age[64], viral fitness and genetic predisposition[65, 66] (CCR5Δ32 deletion). The most important of these is a deletion in the CCR5 coreceptor. Homozygotes for this 32 base pair deletion (CCR5Δ32) do not express the coreceptor at the cell-surface and therefore can only be infected with T-tropic HIV strains that are able to use other coreceptors, such as CXCR4. Heterozygotes for the deletion exhibit significantly lower viral set points and a slower progression to AIDS; these individuals belong to the group of, so called, long-term non-progressors; representing ~ 5% of all HIV –infected patients.

Symptomatic infection eventually occurs in the presence of high viral replication and consequent destruction of the immune system. Although the immune system has the capacity to regenerate it is not unlimited and HIV finally overcomes the immune response. Indeed, once CD4 counts fall to below 200cells/ μl the immune system is sufficiently compromised and patients are at significant risk of contracting many AIDS-defining illnesses, including a number of opportunistic infections (e.g. tuberculosis) and certain neoplasms (e.g. Kaposi's Sarcoma). Above 200cells/ μl, most AIDS-defining illnesses are rare events. Without antiretroviral treatment most patients with symptomatic infection will eventually succumb within 2-3 years.

1.2.1.5 HIV Testing

The laboratory diagnosis of an HIV infection is normally made indirectly by measurement of virus-specific antibodies[67] with most screening tests based on the ELISA principle (enzyme linked immunosorbent assay). HIV infection may also be diagnosed through detection of the virus using branch chain DNA PCR and GenProbe, detecting either intracellular proviral cDNA (complementary DNA) in leucocytes or extracellular HIV-1 RNA in the cell-free compartment. However this approach to viral detection is only relevant in certain situations, such as suspected primary infection or to test babies born to HIV-infected mothers in resource endowed settings.

In all cases, HIV infection can only be confirmed/diagnosed by a reactive (positive) result followed by at least one confirmatory test result. HIV infection should never be diagnosed (or reported to the patient) on the basis of a single reactive screening assay alone. Whereas qualitative testing for viral genome/ virus-specific antibodies serves as a marker of infection, the quantitative detection of HIV RNA in plasma; in copies per millilitre of plasma and the CD4 T-lymphocyte count (number of cells per microlitre of blood) are key prognostic indicators of a patient's viral burden/infectivity status and immunological function, and are routinely used to guide clinical and therapeutic management of HIV-infected patients. HIV RNA is commonly measured by a commercially available RT-PCR kit by Roche, which has a detection limit of 50 copies/ml; hence, in current practice, patients with a pVL below or equal to this value are referred to as 'undetectable' and described as being virologically suppressed.

1.2.1.6 Clinical and laboratory staging of HIV disease

In developing countries like Ghana, the WHO staging system that include clinical, laboratory and a combined clinical /laboratory classifications is used to stage HIV/AIDS in adults and adolescents as shown on Table 1.1A and 1.1B. The clinical markers fall into four stages of prognostic significance and this forms the basis of the WHO clinical staging. In resource limited settings this staging system, which has proven reliable in predicting morbidity and mortality is used to classify clients according their level of immunosuppression, to help prompt clinicians to look out for other disease features when one is present in a stage and to decide when to start cART.

Table 1.1A. The revised WHO clinical staging of HIV/AIDS for adults and adolescents.

Clinical Stage	Clinical conditions	
1^0 HIV Infection	Acute retroviral syndrome	
1	Asymptomatic	
	Persistent generalised lymphadenopathy	
2	Moderate unexplained weight loss <10% of presumed or measured body weight	Recurrent oral ulcerations
	Recurrent upper respiratory tract infections	Papular pruritic eruptions
	Herpes zoster	Seborrhoeic dermatitis
	Angular cheilitis	Fungal nail infections of fingers
3	***a presumptive diagnosis can be made on the basis of clinical signs or simple investigation**	**** a diagnostic confirmatory test is necessary**
	Severe weight loss >10% of presumed or measured body weight	Unexplained anaemia (< 8g/dl), and or neutropenia (<500/mm^3)
	Unexplained diarrhoea for > 1 month	Unexplained thrombocytopaenia (<50 000/mm^3) for > 1month
	Unexplained persistent fever (intermittent or constant)	
	Oral candidiasis	
	Oral hairy leukoplakia	
	Pulmonary tuberculosis diagnosed within the last 2 years	
	Severe presumed bacterial infections e.g. pneumonia, empyema, meningitis, bone and joint infection	
	Acute necrotising ulcerative stomatitis, gingivitis, or periodontitis	
4		**** a diagnostic confirmatory test is necessary**
	HIV wasting syndrome	Extrapulmonary cryptococcosis
	Pneumocystis pneumonia	Disseminated non-TB mycobacterial infection
	Recurrent severe or radiological bacterial pneumonia	Progressive multifocal leukoencephalopathy (PML)
	Chronic herpes simplex infection (orolabial, genital or anorectal) >1month	Candida of trachea, bronchi or lungs
	Oesophageal candidiasis	Cryptosporidiosis
	Extrapulmonary tuberculosis	Isosporidiosis
	Kaposi sarcoma	Visceral herpes simplex infection
	CNS toxoplasmosis	Visceral leishmaniasis
	HIV encephalopathy	Invasive cervical carcinoma
		Lymphoma (cerebral or B cell non-Hodgkin)
		Recurrent non-typhoidal salmonella sepsis
		Any disseminated mycosis such as histoplasmosis,

						coccidiomycosis, penicilliosis Cytomegalovirus (CMV) infection (retinitis or of any organ other than liver, spleen or lymph nodes)

Table 1.1B. Improved WHO Clinical staging.

Laboratory axis			Clinical axis			
Lymphocyte count		CD4 counts	Stage 1 Asymptomatic PGL	Stage 2 Early HIV	Stage 3 Intermediate (ARC)*	Stage 4 Late AIDS
A	> 2000	>500	1A	2A	3A	4A
B	1000- 2000	200-500	1B	2B	3B	4B
C	< 1000	<200	1C	2C	3C	4C

1.2.2 Combination Antiretroviral Therapy (cART)

Combination antiretroviral therapy (cART) involves the simultaneous administration of three or more antiretroviral drugs. Currently there are more than 20 antiretroviral medications from six different mechanistic classes including: the nucleoside/nucleotide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PI), entry/ fusion inhibitors (FIs), CCR5 antagonists and integrase strand transfer inhibitors (INSTIs). These drugs target different stages in the HIV life cycle. In addition there are several novel and experimental antiretroviral drugs including maturation inhibitors, uncoating inhibitors, transcription inhibitors and translation inhibitors at various stages of development.

1.2.2.1 Nucleoside/Nucleotide Reverse Transcriptase Inhibitors (NRTI)

Blockade of the viral reverse transcriptase enzyme was the first attempt to inhibit the HIV life cycle, and in 1987 the first antiretroviral, zidovudine (AZT) was licensed for treatment of HIV infection. The NRTI act as competitive inhibitors of reverse transcriptase. The drugs in this class enter the host cells via endocytosis and require intracellular phosphorylation in order to produce an active triphosphate form. The triphosphate derivatives then act as alternative substrates for the viral reverse transcriptase and compete with physiological nucleosides, since their structure differs by only a minor modification in the ribose molecule. The incorporation of nucleoside analogues ultimately terminates viral DNA replication, as phosphodiester bridges can no longer be built to stabilise the DNA double strand.

The overall tolerability of NRTI is fairly good, with initial side effects being easy to manage. The pill burden is low compared with other drug classes [once daily (*q.d.*) dosing is sufficient for most NRTI], which reduces the potential for non-adherence[68, 69]. There is also a reduced risk of drug interactions, as NRTI predominantly undergo renal excretion and are less likely to interact with drugs metabolised by the hepatic/intestinal cytochrome P450 (CYP450) system. Despite these potential advantages, the NRTI are associated with long-term safety problems, including mitochondrial toxicity caused by their inhibition of mitochondrial DNA polymerase-γ[70].

Two NRTIs remain an integral part of cART and are combined with either an NNRTI or a ritonavir-boosted PI. There are currently seven NRTI available, namely, zidovudine (AZT), didanosine (ddI), stavudine (d4T), lamividine (3TC), tenofovir (TDF), abacavir (ABC) and emtricitabine (FTC). Extensive data support the inclusion of 3TC and/or FTC as one of the two NRTI[71-73]. Single-pill formulations containing two or more NRTI are also licensed to further simplify treatment regimens. Combinations include AZT +

3TC (Combivir), ABC + 3TC (Kivexa), TDF + FTC (Truvada) and 3TC + AZT + ABC (Trizivir).

1.2.2.2 Non-nucleoside Reverse Transcriptase Inhibitors (NNRTI)

The NNRTIs, which include efavirenz (EFV), nevirapine (NVP) and delavirdine (DLV) also possess a high affinity for the enzyme reverse transcriptase. However, unlike the NRTI they do not act as false substrates or require transformation within the cell to form an active metabolite. Instead, the parent form binds non-competitively to the viral enzyme at a specific region known as the NNRTI pocket, located close to the substrate binding site. The resulting complex blocks the active site by allosteric interactions, so that fewer nucleosides can bind, thereby slowing the polymerase reaction significantly.

EFV can cause mild CNS manifestations, including dizziness, somnolence, impaired concentration and nightmares, and is best taken at night. However, these effects are usually transient, only occurring during the initial two to four weeks of therapy, although they can persist in approximately one fifth of patients[74, 75]. NVP can provide an alternative to EFV in pregnant women or patients with baseline mental health disorders[76]. However, NVP is known to cause elevation of liver enzymes in up to 16% of patients which can result in severe hepatotoxicity, and must be administered under specific CD4 criteria. Women with good immune status particularly those with CD4 $>350/mm^3$ appear to be more prone to this effect. DLV is rarely prescribed due to its high pill burden and is not licensed in Europe and in Africa.

Overall, the NNRTIs are well tolerated and have relatively long half-lives permitting simple dosing. Their one major drawback however is the low genetic barrier to resistance, in which single amino acid substitutions (K103N and Y188C) are sufficient to confer NNRTI cross-resistance[77] necessitating a swift but decisive change in treatment regimens. In addition, a risk of drug-drug interactions with NNRTI is high as both EFV and NVP are metabolised by the CYP450 system and cause induction (NVP) or both induction and inhibition (EFV) of CYP3A4 and CYP2B6 and can therefore impact the metabolism of concurrent drugs[78] which will later be discussed under Section 1.2.7.3.ii. of this literature review.

Second generation NNRTI: due to the genetic frailty and potential for cross-resistance, a second generation of NNRTIs, including etravirine (ETV) and rilpivrine (RPV), has been developed. Both are flexible diarylpyrimidine compounds which possess favourable binding interactions toward both the wild-type and mutant HIV, including virus harbouring the common K103N mutation which confers resistance to all first generation NNRTI[79]. Specifically, structural studies have shown that the diarylpyrimidine can adapt to changes in the NNRTI-binding pocket by binding in at least two conformationally distinct modes: (a) within a given binding mode, torsional flexibility of these analogues permits access to numerous conformational variants and (b) the compact design of the analogues permits significant repositioning and reorientation within the pocket. In 2008, the US Food and Drug Administration (FDA) and the European Medicines Agency (EMEA) granted accelerated approval of ETV based on data from the phase III DUET_1 and DUET_2 studies, in which patients in the ETV arm achieved better virologic and immunologic responses compared with the placebo when combined with an optimised background regimen of RTV boosted DRV with or without the fusion inhibitor enfuvirtide[80-82]. Similarly RPV, has been shown to be effective in phase III trials for use in both ART-naïve[83, 84] and ART-experienced[85] patients.

1.2.2.3 Protease Inhibitors (PI)

The first protease inhibitor, saquinavir (SQV), was introduced in December 1995. Since then the PIs have remained an essential component of cART, particularly among treatment experienced patients. The PIs target the HIV protease enzyme, responsible for cleaving large HIV precursor proteins into infectious viral particles that are able to bud through the host cell membrane. The drugs bind tightly to a peptide moiety within the catalytic pocket of the protease enzyme and prevent it from cleaving HIV polyproteins into the functional proteins. As a result, the virus cannot mature and non-infectious viral particles are produced.

The PI undergo extensive CYP450 mediated metabolism via CYP3A4 and to a lesser extent by CYP2D6 and CYP2C19, which renders them prone to variable pharmacokinetics and extensive drug-drug interactions when given in combination or with other concomitant medications[86]. Moreover, they can variably affect their own metabolism through induction and inhibition of these enzymes[87-89]. All PIs inhibit CYP3A4, with ritonavir (RTV) being the most potent and is extensively used at sub-therapeutic doses (100mg or 200mg) as a pharmacokinetic enhancer to “boost” the plasma concentrations of concomitant PI[90]. Boosting with RTV increases drug exposure and/or prolongs the elimination half-life, allowing for a reduction in pill burden and dosing frequency, which, in turn improves patient adherence and limits the development of resistance.

The RTV-boosted PIs recommended for initial treatment in naïve patients include atazanavir (ATV), lopinavir (LPV), amprenavir [APV; administered as the pro-drug fosamprenavir (FPV)] and SQV. In addition, boosted darunavir (DRV), which is active against multi-drug resistant strains of HIV-1 and shown to be superior to LPV in patients with HIV RNA > 100,000 copies/ml[91] has been approved for initial treatment in early 2009. Kaletra (LPV/r) is the only licensed PI to contain a fixed booster dose of ritonavir and is the only PI available in Ghana and is reserved as a component of second line therapy for treatment failures.

Boosted PIs have a higher genetic barrier to resistance and exhibit improved immunological responses compared to the NNRTI[92]. However, most PIs (excluding ATV) are commonly associated with undesirable effects on lipid metabolism, including hyperlipidemia [an increase in total and low density (LDL) cholesterol and triglyceride levels, which poses an increased cardiovascular risk] and lipodystrophy (redistribution of body fat, often with fat wasting at the extremities and around the face, and fat deposition around the trunk).

1.2.2.4 Entry inhibitors

For treatment experienced patients harbouring resistance virus and those failing multiple regimens, antiretroviral drug combinations have become increasingly complex and in recent years new and more potent agents have been introduced which possess activity against both wild-type and resistant strains.

Inhibition of viral entry is a promising target for therapeutic intervention, since neither NRTI/ NNRTI nor PI prevent viral entry. In theory all stages of viral entry can be inhibited, including, attachment or binding to the CD4 receptor, binding to coreceptors and fusion of the virus and host membranes. Hence entry inhibitors can be divided into three classes: *attachment inhibitors, coreceptor antagonists* and *fusion inhibitors*.

Attachment inhibitors

These agents interfere with the interaction between the HIV glycoprotein gp120 and the CD4 receptor, which is the first step towards viral entry into the target cell. Attachment inhibitors are very heterogeneous class because sites on the CD4 receptor as well as the binding site for gp120 can be blocked; both are currently being investigated. Compounds under investigation include ibaluzimab (formerly TNX-355)[93], a humanised monoclonal antibody that binds to the domain 2 of the extracellular portion of the CD4 receptor as well as BMS-663068 [94] and BMS-488043[95] which binds to the CD4 binding pocket of the viral envelope gp120 to interfere with CD4 binding. These are in the early stages of development.

Co-receptor antagonists

In addition to the CD4 receptor, HIV also requires co-receptors to gain entry into a target cell. Co-receptor antagonists block either CCR5 or CXCR4 in a similar way to endogenous chemokines, whose structure they partially resemble. The CCR5 antagonists, in particular, are in the later stages of development. Maraviroc (MVC) is the first licensed co-receptor (CCR5 receptor) antagonist and was approved in August 2007. It has activity specifically against CCR5-trophic HIV by preventing the virus from engaging with the CCR5 co-receptor located on the host cell membrane[96]. MVC is efficacious in patients with R5 virus, failing other antiretroviral classes[97]. However, it is not active against CXCR4-tropic and mixed or dually-tropic strains, which become increasingly dominant in the later stages of HIV infection. Thus, an initial determination of co-receptor tropism is necessary prior to initiating therapy. Furthermore, it is possible that blocking one type of co-receptor may itself induce a switch in viral tropism. There are also long-term safety concerns of blocking a human receptor, as opposed to a viral target. Initial data from a study evaluating MVC in treatment naïve patients (MERIT) suggest that it was inferior to standard-of-care EFV when using <50 copies/ml cut-off[98]. However in a subsequent analysis, which used a more sensitive tropism testing assay than the one originally used and retrospectively excluded patients with non CCR5-tropic HIV-1 infection, MVC demonstrated noninferiority to efavirenz on primary virological end-points[98]. In treatment-naïve patients, vicriviroc administered with dual NRTIs in treatment-naïve subjects with HIV-1 infection has increased rates of virologic failure compared with efavirenz plus dual NRTIs[99].

Fusion inhibitors

Enfuvirtide (T-20) is the only fusion inhibitor approved for clinical use, although other compounds of this class are currently in development. T-20 binds to an intermediated structure of the gp41 protein to prevent the formation of the HR-1:HR-2 complex and ultimately fusion of HIV with the target cell. It is a large peptide molecule (36 amino acids) and is homologous to HR-2 [100]. A practical limitation to clinical use however is that, because of its size, T-20 has to be administered via subcutaneous injection[101]. T-20 is used primarily in patients harbouring multi-resistant strains, for the so-called ‘salvage therapy’ in cases where other treatment options have been exhausted. Improved efficacy by incorporating T-20 into an optimised HAART regimen in extensively pre-treated patients was demonstrated by the TORO_1 and TORO_2 studies, respectively [102-104].

1.2.2.5 Integrase Inhibitors

The viral enzyme integrase, which is encoded by the HIV pol gene, is involved in the integration of viral DNA into the host genome; rendering it a key pharmacological target[105]. Moreover, the enzyme is unlikely to be present in human cells. Integration of viral DNA is a stepwise process, all of which can be theoretically inhibited. Raltegravir (RAL) is the first integrase inhibitor to be licensed by the FDA and EMEA in 2007. The drug specifically inhibits the strand transfer step in the integration process, by preventing the docking and irreversible binding of the hydroxyl ends of viral DNA to the phosphodiesterase bridges of the host DNA. RAL is efficacious against both R5 and X4 tropic viruses and has demonstrated potent antiviral activity in multi-drug experienced patients, as presented in the BENCHMARK trial[106]; although, due to concerns over genetic frailty, it needs to be supported by other active agents. Unlike most antiretrovirals, RAL is eliminated primarily by UDP-glucoronyltransferase (UGT1A1) mediated glucoronidation, and thus has less potential for CYP-related drug interactions[107]. An additional integrase inhibitor, elvitegravir[108], also a strand transfer inhibitor, is in the late stages of clinical development, but has yet to be approved. Elvitegravir is currently boosted with RTV but a new boosting agent, GS9350, is currently been trialled. GS9350 does not have anti HIV activity and is well tolerated[109]. Recently, elvitegravir (EVG) co-formulated with the CYP3A4 inhibitor cobicistat (COBI), emtricitabine (FTC) and tenofovir (TDF) in a single tablet given once daily was shown to be virologically non-inferior to once daily EFV/FTC/TDF at 48-weeks in ART-naïve patients[110] showing that if regulatory approval is given, EVG/COBI/FTC/TDF (Quad) would be the only single tablet, once-daily, integrase-inhibitor-based regimen for initial treatment of HIV infection.

In summary the over past 25 years tremendous strides have been made in antiretroviral drug development. Data from the MOTIVATE (maravaroc), BENCHMARK (raltegravir), TORO (T-20) and DUET (etravarine) studies have shown clear benefit of adding a fully active drug to an existing optimised regimen in multi-drug experienced patients. Therefore, it is hoped that with access to these additional classes, patients with extensive multi-drug resistance can realistically achieve the same virologic and immunologic responses seen in naïve patients. However these novel antiretroviral drug classes are expensive and may become accessible to the majority of people living with HIV in resource limited settings in the coming years.

1.2.3 Initiating first line combination antiretroviral therapy

1.2.3.1. When to start

One of the most crucial decisions is when to initiate antiretroviral therapy (ART), as this can influence a patient's long term response to treatment. Most of the early treatment guidelines recommended that treatment be delayed until the CD4 cell count had fallen below 200 cells//µl. However over time, treatments have improved and the number of treatment options available to patients has increased. Current international guidelines state that all symptomatic and asymptomatic patients with a CD4 count less than 350 cells/µl according to the BHIVA guidelines[111]. According to the recent WHO guidelines, all adolescents and adults including pregnant women with HIV infection and CD4 counts of $\leq$ 350 cells/mm^3, should start ART, regardless of the presence or absence of clinical symptoms. Those with severe or advanced clinical disease i.e WHO clinical stage 3 or 4) should start ART irrespective of their CD4 cell count[112].

Asymptomatic patients with CD4 counts >500 cells/μl have a low short-term risk of disease progression; thus it is recommended that treatment in these subjects should be deferred in the majority of cases. The question of whether to initiate ART in asymptomatic patients with CD4 counts >350 cells/μl (and even > 500 cells/μl) is uncertain. Recent cohort studies, most significantly, SMART and NA-ACCORD, suggest that early initiation of therapy [>350 cells/μl[113] and >350 and >500 cells/μl[114] improved survival rates and was associated with a reduced incidence of non-AIDS malignancies and cardiovascular diseases compared with deferred therapy. Indeed, previous concerns with respect to starting therapy early has been based on the toxicity risks (for example, metabolic complications) associated with long-term antiretroviral therapy. However, because simpler, less toxic and more tolerated agents are now available, as well as an increased number of options in the case of virologic failure, early initiation of therapy is becoming an increasing viable consideration at least in the resource-endowed settings. The Strategic Timing of Antiretroviral Treatment (START) trial due to report in 2015 will provide the first randomised evidence of whether immediate initiation of treatment in patients with CD4 cell counts more than 500 cell/μl is superior to delaying initiation of HAART until the CD4 cell counts falls below 350 cells/μl.

1.2.3.2. What to start with

The preferred options for adults and adolescents in the 2010 WHO recommendations for starting first line cART in developing countries such as Ghana for patients with HIV-1 infection include either AZT or TDF + 3TC or FTC with either EFV or NVP. The backbone of AZT+3TC is avoided in patients with moderate to severe anaemia.

Previously D4T+3TC was recommended as part of first line therapy, but D4T although relatively inexpensive is being phased out in most treatment programmes in developing countries to avoid the disfiguring, unpleasant and potentially life-threatening toxicity.

In comparison the current 2012 BHIVA guidelines recommend for treatment naïve patients, a combination of three or more antiretroviral agents: an NNRTI or a RTV-boosted PI in combination with a dual NRTI backbone (preferably containing either FTC or 3TC). These recommendations are summarised in Table 2.2. Since ART is life-long, it is crucial that initial drug regimens are tailored towards an individual patient's needs, taking into account any concurrent illnesses and concomitant medications, in order to achieve the maximum potency and tolerability, and avoid long-term complications and possible drug interactions.

Table 1.2 Current (2012) British HIV Association (BHIVA) recommendations for initial treatment of HIV infection.

	Preferred	**Alternative**
NRTI backbone	Tenofovir and emtricitabine	Abacavir and lamivudine[1,3]
Third agent	Atazanavir/Ritonavir Darunavir/Ritonavir Efavirenz Raltegravir	Lopinavir/Ritonavir Fosamprenavir/Ritonavir Nevirapine[2] Rilpivirine[3]

1. Abacavir is contraindicated if HLA B*5701 is positive

2. Nevirapine is contraindicated if baseline CD4 is greater than 250/400 cell/µl in women/men.

3. Use recommended only if baseline viral load less than 100,000 copies/ml: rilpivirine as a third agent, Abacavir+Lamivudine as NRTI back bone.

1.2.4 Factors influencing response to cART

The primary goal of ART in naive patients is to achieve an undetectable pVL of <50 copies/ml within the first 4-6 months of initiating treatment[111]. If virologic suppression is not attained, or the log reduction in pVL from baseline is insufficient, patients are described to have ‘failed’ therapy. If antiretroviral drug classes fail virologically and there are little or no remaining options for the patient concerned, clinicians must take decisive action and explore all possible avenues of treatment; this therapeutic approach is often referred to as ‘salvage’ therapy. In the past few years, with the advent of new and more potent antiretroviral classes which possess activity against an array of drug resistant strains, the outlook for these patients is increasingly promising.

An individual’s response to ART is dependent on many confounding variables, which relate to the patient or ‘host’, the virus and the drug(s) administered. Thus, treatment failure can occur due to physiological, pathological, genetic and behavioural factors, and pharmacologically, due to poor pharmacokinetics, drug interactions, a lack of treatment efficacy or the development of viral resistance; all of which are explored further in the following sections.

1.2.4.1. Viral factors

Drug resistance

Treatment failure is often indicated by a high pVL in a patient on ART and it implies that the virus has acquired resistance to a drug, or a whole drug class (cross-resistance). Resistance occurs when viruses acquire mutations that render them slightly different from the original wild- type population. The development of resistance in HIV is rather ubiquitous because of both the rapid and error prone replication, as the reverse transcriptase enzyme does not contain a DNA proofreading stage like other retroviruses[115]. Specifically, mutations that confer drug resistance accumulate when viral replication occurs in the presence of selective pressure from antiretrovirals and /or immune response. The potential for selection of drug resistant strains is, therefore, substantially higher in the presence of suboptimal antiretroviral concentrations, when the replicative capacity of the virus becomes greater. For this reason, effective monitoring of antiretroviral concentrations may be a viable tool for evaluating or predicting the risk of resistance in patients, particularly those with high pVL and/or suspected adherence issues.

However, many drug resistant mutations which emerge during antiretroviral treatment have a detrimental effect on viral fitness and replication. In general, mutations conferring resistance to the NRTIs and NNRTIs do not reduce viral fitness to the same extent as those conferring resistance to the PIs. For example, the D30N primary mutation for nelfinavir (NFV) reduces the replicative capacity of the virus but does not impact the efficacy of other PI[116]. By contrast, the single point mutation K103N is enough to cause cross-resistance to all first generation NNRTI and does not change the replicative capacity of HIV [115, 117], so a prompt change in therapy is vital to avoid NNRTI exclusion, and preserve future options. Furthermore, in the era of cART, the transmission of resistant strains is an emerging problem which has clear implications for long-term treatment options. As a result, where available newly diagnosed patients are screened for resistant strains prior to initiation of therapy in the resource-endowed settings.

1.2.4.2. Pharmacological factors

Pharmacological factors that may incur therapeutic failure include poor drug pharmacokinetics, inadequate potency and a low genetic barrier to resistance, unfavourable toxicity profiles and poor penetration of antiretrovirals into viral sanctuary sites.

Pharmacokinetics

Pharmacokinetics is the area of pharmacology which describes the **A**bsorption, **D**istribution, **M**etabolism and **E**limination (ADME) of drugs by physiological systems in the body. In the field of HIV, acquiring and maintaining antiretroviral concentrations within the systemic circulation is essential in ensuring that therapeutic drug concentrations reach their local receptor site (i.e. within CD4+ cells) in order to exert the desired pharmacological response.

Most significantly, the NNRTIs and PIs have well-defined pharmacokinetic/ pharmacodynamic (PK/PD) and pharmacokinetic/toxicity relationships in which, systemic (plasma) drug levels have been shown to correlate with observed virologic response, or to independently predict the risk of treatment failure/success[118 - 121] or toxicity[122 - 124]. Thus, characterisation of the relationship between antiretroviral pharmacokinetics (systemic exposure or a single concentration) and drug response (beneficial and/or adverse) is key to the selection of an optimal dose for a drug, understanding inter and intra-subject variability, and to design strategies to optimise response and tolerability whilst avoiding unwanted toxicity.

Furthermore, it is essential that antiretroviral concentrations remain above a so-called minimum effective concentration (MEC) in order to ensure adequate potency and avoid viral rebound or development of resistance. On the other hand, concentrations must not be so high as to cause unwanted toxicity [125,126]. These concentration-based therapeutic cut-offs (MEC) which are well defined for most NNRTIs and PIs [127] are utilised in both prospective and observational pharmacokinetic studies, and in routine therapeutic drug monitoring (TDM) in resource endowed settings. When evaluated alongside virological (pVL) and immunological (CD4 counts) markers, they may aid in the interpretation of an individual's response to therapy enabling physicians to make more rational or 'evidence- based' decisions regarding dose adjustment in case of sub-therapeutic concentrations or an unsuppressed pVL. Nevertheless, these values serve only as estimates, and because they are derived primarily from accumulated pharmacokinetic data obtained from controlled trials, they may not reflect 'real-life' clinical situations, such as the effects of drug interactions, co-infections and pregnancy as well as differences in age, race and disease status upon overall drug exposure. Indeed, in reality, there is higher inter and intra-individual variation in antiretroviral plasma concentrations, even in patients receiving equivalent dose[126]. The foundations for such an effect is not fully understood, although it is possible that pharmacokinetic factors, such as differences in drug absorption, metabolism and distribution, along with external patient or 'host' influences (as discussed below) could contribute to the underlying variability in antiretroviral concentrations seen in HIV-infected patients.

All antiretrovirals, with the exception of T-20, are administered orally; thus, in order to reach the systemic circulation and be distributed throughout the body, they must be absorbed through the enterocytes and the gut wall. The rate and extent of drug absorption is highly dependent upon an agent's physicochemical properties, such as its lipophilicity (as determined by its partition or distribution coefficient; logP/logD) and its solubility (dissociation constant; pKa); but may also be regulated by gastrointestinal motility, gastric and gastrointestinal pH and blood flow which, in turn, can be affected by food (mainly fat) intake, pregnancy, disease states and circadian differences. Most antiretrovirals, excluding the NRTI, which are eliminated renally upon reaching the liver, undergo phase I biotransformation via oxidative reactions involving the CYP450 superfamily, followed by phase II conjugation reactions before being eliminated in urine or bile. The primary routes of metabolism and elimination of antiretroviral drugs are summarised in Table 1.3, respectively. In addition, prior to passage through the liver, agents may also be subjected to metabolism and cellular efflux by CYP450 enzymes and transporters present in the gastrointestinal tract. The removal of drugs via these processes is termed 'first pass metabolism', and can significantly reduce drug oral bioavailability; that is, the fraction of unchanged drug reaching the systemic circulation upon administration of an oral dose.

Table 1.3 Metabolism of antiretroviral drugs

Drug	**Metabolic pathway**
Nucleoside and Nucleotide Reverse Transcriptase Inhibitors (NTRI)	
Zidovudine (AZT)	Glucuronidation (glucuronyltransferase UGT2B7), renal excretion (glomerular filtration and active tubular secretion)
Didanosine (ddI)	Renal excretion (glomerular filtration and active tubular secretion)
Stavudine (d4T)	Renal excretion
Lamivudine (3TC)	Renal excretion
Tenofovir (TDF)	Renal excretion (glomerular filtration and active tubular secretion)
Abacavir (ABC)	Hepatic metabolism (glucuronyltransferase and alcohol dehydrogenase), subsequent renal excretion of metabolites
Emtracitabine (FTC)	Renal excretion, oxidation and glucuroconjugation (<10%)
Non-Nucleoside Reverse Transcriptase Inhibitors (NNRTI)	
Efavirenz (EFV)	P450 (CYP3A4, CYP2B6)
Nevirapine (NVP)	P450 (CYP3A4, CYP2B6)
Etravirine (ETV)	P450 (CYP3A4, CYP2C9, CYP2C19)
Protease Inhibitors (PI)	
Fosamprenavir (FPV)	P450 (CYP3A4)
Atazanavir (ATV)	P450 (CYP3A4)
Indinavir (IDV)	P450 (CYP3A4)
Lopinavir/Ritonavir(LPV/r)	P450 (CYP3A4)
Nelfinavir (NFV)	P450 (CYP3A4, CYP2C9, CYP2C19, CYP2D6)
Ritonavir (RTV)	P450 (CYP3A4, CYP2C9)
Saquinavir (SQV)	P450 (CYP3A4)
Taprinavir (TPV)	P450 (CYP3A4)
Darunavir (DRV)	P450 (CYP3A4)
Entry inhibitors	
Enfuvirtide (T-20)	Catabolism to amino acids
Maraviroc (MVC)	P450 (CYP3A4)
Integrase Inhibitors	
Raltegravir (RAL)	Glucuronidation (UDP-glucuronyltransferase, UGT1A1)

The NNRTI and PI undergo CYP450 mediated metabolism via CYP3A4 and to a lesser extent by CYP2B6, CYP2D6 and CYP2C19 which renders them prone to variable pharmacokinetics and extensive drug-drug interactions when given in combination or with other concomitant medications[128]. The PIs, in particular, have unfavourable pharmacokinetic profiles, due to their extensive first pass metabolism; and are therefore characterised by a low oral bioavailability and short elimination half-lives, which necessitates frequent dosing and a high pill burden. The PI are also substrates, inhibitors and inducers of P-glycoprotein (P-gp), an ATP-dependent transmembrane glycoprotein which functions as an efflux pump for a wide variety of compounds and shows a degree of overlap in substrate specificity with CYP3A4[129-131]. Furthermore, the organic anion transport polypeptides (OATPs: influx transporters)[132] and multidrug resistance associated proteins (MRP1/MRP2) are also involved in the disposition of certain PI[133-137]. For this reason, and as previously described, the PI are combined with sub-therapeutic RTV doses (100mg or 200mg) as a means of ‘boosting’ drug levels. RTV elevates concurrent PI plasma levels via inhibition of hepatic and intestinal CYP3A4, the major enzyme involved in the biotransformation of PIs. In addition, it may also inhibit the efflux transporter P-gp, for which PI possess varying affinity, and hence limit cellular drug efflux[138, 139]. Boosting variably results in an increase in the minimum or trough plasma concentration (C_{trough}), maximum concentration (C_{max}) and the elimination half-life ($t_{1/2}$), although the overall effect depend on the metabolic properties of the concomitant PI. Interestingly, PIs that are boosted by RTV may themselves affect RTV pharmacokinetics. For instance, while slightly higher than expected concentrations of RTV have been observed following the administration of 100mg of RTV with ATV, slightly lower concentrations have been observed in the presence of LPV, FPV, and TPV[140]. This may be due to differential effects (induction versus inhibition) that the PI exert on CYP3A4 activity, since this isoenzyme is also responsible for RTV metabolism itself.

Once a drug reaches the systemic circulation it is distributed within the blood and extravascular tissue compartments depending on its lipophilicity and affinity for plasma proteins. Since only unbound (free) drug can penetrate cell membranes, distribution will continue until the concentrations of the unbound drug in plasma and tissue water reach equilibrium. The apparent volume of distribution (V_d) is a measure of the extent of a drug's distribution outside of plasma once this equilibrium is reached. Many of the antiretrovirals have moderate to relatively large V_d (>0.7L/kg body weight), which means that most of the drug within the body actually resides outside of plasma, within the tissue compartment[106].

The NNRTI and PI are highly (80-99%) bound to plasma proteins, with the exception of NVP and IDV which are only moderately (~60%) bound[141]. The NNRTIs are weakly acidic compounds and preferentially bind to human serum albumin (HSA), whereas the PI are weakly basic and predominantly bind to the acute phase protein alpha-1 acid glycoprotein (AAG).

Sanctuary sites

HIV is also present outside the blood compartment in sanctuary site including the male and female genital tract (semen and cervicovaginal secretions), lymphoid tissue and the CNS (cerebrospinal fluid; CSF), so called because they act as regions within the body scarcely accessible by antiretroviral drugs. These anatomical sanctuary sites should be distinguished from cellular sanctuary sites (or viral reservoirs; which represent the pool of latently infected resting CD4+ T-cells containing non-replicating integrated HIV provirus) [142, 143] as virus is actively replicating in these sites, thus viral rebound can occur if the antiretroviral therapy is discontinued. Adequate penetration of active drug within such areas has implications in preventing sexual and vertical (mother-to-child) transmission of the virus and in the incidence of HIV associated neuropathies. Differential penetration of antiretrovirals into sanctuary sites is attributed, at least in part, to their affinity for drug transporting proteins and binding to plasma proteins. The PIs generally show poor penetration into the brain and genital tract. For example concentrations of LPV in semen were shown to approximate only 2-3% of that in plasma[144] and ATV levels in CSF were less than 1% of concentrations in plasma, respectively[145].

1.2.4.3. Host factors

External and internal influences relating to the patient or 'host' may further contribute to the observed inter-subject variation in drug concentrations and response to treatment. Such factors can be psychological, such as the patient's life-style and adherence to therapy, pathological (the stage of HIV infection and the presence of concomitant diseases particularly co-infections), or physiological (age and gender-related differences, or changes in body weight and composition).

Adherence

Patient non-adherence to therapy has been cited as one of the main causes of treatment failure in HIV patients[146]. Past studies have demonstrated that >95% adherence is essential for achieving viral suppression and treatment success[147, 148]. If the drugs are not taken appropriately, plasma concentrations may not be maintained above their therapeutic thresholds, resulting in suboptimal viral suppression and therefore an increased risk of viral rebound or development of resistance. Furthermore, non-compliant patients may compromise the treatment of others, as they have a potential for transmission of resistant viruses.

ART is for life, and continuous adherence to therapy can be challenging even for the most willing of patients given the high pill burden of some regimens and necessity for frequent and accurately timed dosing. Moreover, the treatment of, what is essentially in newly infected patients, an 'asymptomatic' disease, means patients may be less willing to adhere to their medication in the case of unwanted side effects. For example, the lipid abnormalities associated with the PI have often led to treatment discontinuation[149].

Previous studies have demonstrated that the PI-based regimens in particular require greater than 90% adherence to achieve durable viral suppression, most likely due to their short elimination $t_{1/2}$ [148, 150 - 152]. Also, accumulating evidence suggests that adherence is higher and dose timing accuracy improved in patients receiving once daily (*q.d.*) compared to twice (*b.i.d.*) and thrice (*t.i.d.*) daily regimens[153, 154]. However, irrespective of improved adherence, when considering antiretroviral forgiveness (which refers to the ability of a dose of a drug to maintain adequate concentrations in the face of late or omitted subsequent doses), the relative benefit of *q.d.* regimens is by no means irrefutable, since a single missed *q.d.* dose may give rise to a higher risk of suboptimal concentrations as the drug can be absent over a 24 hour period. As antiretroviral forgiveness is dependent on both potency (genetic barrier to resistance) and $t_{1/2}$, most

NNRTIs and NRTIs approved for *q.d*. dosing are suitable based on their prolonged $t_{1/2}$; however there is a degree of uncertainty regarding the use of PI-based q.d. regimens[155]. Indeed in the FOTO study by Cohen *et al*[155] to study the feasibility of intermittent antiretroviral combination therapy taken five days a week, with two consecutive days off, found that for regimens containing drugs with long $t_{1/2,}$ such as the NNRTI (EFV), missed doses had no detrimental effect upon virologic suppression; whereas, the strategy appeared much riskier for patients receiving PI-based regimens. In fact, 5 of the 6 individuals on LPV/r or SQV/r had sub-therapeutic drug concentrations at the end of the second day off therapy; suggesting that despite the presence of the RTV, LPV and SQV plasma concentrations decline rapidly, and may therefore be comparatively less 'forgiving' than the NNRTI[155].

As the issue of patient adherence is continually being addressed, the previous consensus that almost 100% adherence is paramount for achieving virologic success[147, 148] may no longer hold true. In recent years, the introduction of drugs with longer $t_{1/2}$, higher genetic barriers to resistance and improved pharmacokinetic properties enable greater flexibility in dosing and are likely to be more forgiving in the case of missed doses. Furthermore, the availability of dual and triple NRTI combinations, the boosting of PI and the development of new formulations of existing drugs with improved oral bioavailability, have all helped to reduce pill burden and improve the convenience of therapy. Indeed, one of the most significant steps forward in HIV therapy has been the approval of the first fixed-dose formulation (Atripla) which contains 2 distinct antiretroviral classes, EFV, FTC + TDF, and allows for one tablet once daily dosing for virologically suppressed patients.

Gender

Despite women making up nearly 50% of all people living with HIV worldwide and almost 61% of those infected in Sub-Saharan Africa (UNAIDS/WHO 2007), they are underrepresented in clinical programs. There are number of reasons for this including the risk of pregnancy (and teratogenicity) in women of child-bearing age, unwanted interactions from concurrent drugs (e.g. oral contraceptives) and concern over certain social responsibilities (e.g. child care) which may have a detrimental effect on a woman's ability to adhere to the study medication and present regularly to clinic.

Several studies have described differences between men and women in their response to ART and drug pharmacokinetics. Although there is no apparent effect of gender on HIV progression (both in the presence and absence of ART) [156, 157] in developing countries, there is evidence to suggest better clinical outcomes for women than men[158] but women are seemingly more prone to adverse effects from antiretroviral treatment and have higher drug exposure[159-162]. Some evidence exists for gender-related differences in the pharmacokinetics of the PIs. For example, SQV, ATV and IDV plasma concentrations were shown in a number of studies, to be notably higher in women[163 - 165]; although other studies observed no gender-related differences in LPV and IDV plasma concentrations, respectively[166, 167].

It is likely that differences in the level of drug exposure between males and females are primarily driven by differences in body weight and composition. Indeed, reduced body weight alone in females may independently account for the majority of gender-related differences in drug levels, response and toxicity, because crucially the dosing of antiretrovirals in HIV-infected individuals is not adjusted for body weight. Women also possess a relatively higher amount of adipose tissue, a lower skeletal muscle mass and a higher content of body fat than men. Increased fat distribution can lead to a greater accumulation of lipophilic compounds within the tissue compartment and therefore an increased volume of distribution, which, depending on drug clearance can result in an increased $t_{1/2}$ and potentially a prolonged pharmacological effect in women[168]. Additionally, changes in drug metabolism, through differential expression and activity of CYP450 enzymes and influx/ efflux transporters, and modulation of these systems by fluctuating reproductive hormones and the presence of concomitant medications, may also impact the sex-related differences in drug exposure. Indeed, some gender related differences in the expression and activity have been reported for CYP2B6, CYP3A4 and P-gp, with lower expression in females respectively[169]. However, the likelihood of identifying a definitive genetic or CYP-mediated effect is slim given the difficulties in adjusting for multiple confounders (e.g. age, weight, ethnicity, disease status etc). Finally, social and behavioural factors may also determine differences in patient adherence and treatment discontinuation between sexes.

Age (Children and adolescents)

It is estimated that approximately 15% of all HIV-infected individuals are children[1], but the vast majority in the developing countries lack access to cART, which can drastically reduce morbidity and mortality[170]. Furthermore, of the 25 antiretroviral drugs currently approved by the EMEA for use in the treatment of HIV-infected adults and adolescents, only 16 of these drugs are approved for use in children. Like adults and adolescents, children should receive a dual NRTI backbone plus a third potent agent from a different class; either an NNRTI or a RTV- boosted PI[171]. However, there are many challenges involved in treating HIV-infected children, including an uncertainty about when to start treatment, the need for paediatric formulations, a lack of pharmacokinetic studies on existing and new drugs and incomplete dosing guidelines[172].

1.2.5. The therapeutic efficacy of efavirenz (the evidence)

The efficacy of efavirenz compared with other classes of antiretrovirals has been established in numerous randomised controlled trials and observational studies in cART-naïve patients as well as in treatment-experienced patients. Because one cardinal aim of this dissertation was to compare the effectiveness of efavirenz-based cART with nevirapine-based cART over the long-term, the review under this section begins by presenting evidence of treatment effectiveness of efavirenz-based cART compared with nevirapine based cART from the high-income compared with the middle-to-low income countries. This is followed by review of evidence of efficacy of efavirenz compared with other classes of antiretrovirals in combination therapy focusing only on data from naïve patients since the patients in the cohort presented in this dissertation were ART naive.

1.2.5.1 comparison of efavirenz with nevirapine

1.2.5.1.1. Evidence of therapeutic efficacy from randomised controlled and observational studies: Few randomised studies have been performed to compare efavirenz with nevirapine, the only other NNRTI currently licensed for use as first-line therapy. The 2NN study, is the first large, randomised, placebo- controlled trial of nevirapine and efavirenz using a stavudine-lamivudine backbone. At 48 weeks, 70% of patients treated with efavirenz achieved an HIV-1 RNA below the level of detection compared to 65% of the patients treated with the standard dosage of nevirapine. Thus nevirapine did not meet the criteria for non-inferiority as compared with efavirenz[4]. A recently published Cochrane meta-analysis of data from 7 randomised controlled-trials[31] involving 1,688 participants concluded that there were no critical differences between efavirenz and nevirapine in levels of virological suppression except for differences in side effects. Compared with nevirapine given once daily, there were more CNS side effects in the efavirenz arms while the risk of transaminasaemia (on both NVP 200mg twice daily and NVP 400mg once daily) and neutropaenia (on NVP 200mg twice daily) was commoner on nevirapine arm. There were higher discontinuation rates in the EFV arm when compared to the NVP 400mg daily but EFV was slightly less likely than twice daily NVP to be associated with development of antiretroviral resistance. It is noteworthy that the 7 experimental studies included in the meta-analysis varied greatly in the length of follow-up time, cut-off point for undetectable viral load, dosage of NVP and study setting and sub-group analysis did not take NRTI backbones which is a major determinant of virological success and treatment failure[5] into account.

Large observational studies have reported superior virological, immunological and clinical outcomes with efavirenz over nevirapine[5-19]. In a recent study, involving 14,857 patients from North American and European cohorts in the HIV-CAUSAL collaboration, efavirenz was shown to be associated with lower mortality, lower incidence of AIDS-defining illnesses, a larger increase in CD4 count at 12 months and a smaller risk of virologic failure at 12 months compared with nevirapine[20]. In the ART-CC cohort, nevirapine initiation was associated with an adjusted OR for 24-week virological failure of 1.87 (95% CI 1.58 - 2.22) versus efavirenz[173]. Furthermore, nevirapine use was associated with a significantly higher incidence of AIDS events or death over 2 years, compared with efavirenz.

Overall, a systematic examination of evidence of data from 24 observational studies from low-, middle- and high-income countries showed somewhat different results from those from experimental studies[31]. Observational studies conducted in low- and middle-income countries generally favoured EFV over NVP in terms of virologic suppression[7, 8, 14, 15] but favoured NVP over EFV for immunological response[7,9]. In contrast, studies conducted in high-income countries found a more heterogeneous result in terms of virological suppression[5, 16-18] and favoured EFV over NVP in terms of immunological response[17, 174]. Furthermore, a single study by Braithwaite et al.[17] in a high-income country found that EFV was superior to NVP in terms of completion of the initial course of therapy without switching but four studies from low- to middle-income countries found mixed results[6, 8, 175, 176]. The reported rates of severe adverse events were comparable in low- and middle-income countries[6-12, 177] compared to those in high-income countries[13, 24, 178, 179]. Efavirenz resistance mutations were significantly more common in stably treated patients receiving nevirapine than efavirenz (OR 2.73; 95%CI 1.62-4.62; $p<0.001$)[180] . The most likely explanation for these conflicting findings is the uncontrolled bias inherent in observational studies, but the findings may also indicate a subtle difference between the two drugs, which has not been captured in trials.

1.2.5.1.2. Toxicity of efavirenz compared with nevirapine

Skin rashes, neuropsychiatric events and hepatotoxicity are the principal adverse drug reactions associated with the use of nevirapine and efavirenz[4, 181]. Their individual propensities to cause these adverse events differ, with nevirapine showing a higher risk of cutaneous and hepatic reactions, and efavirenz a higher risk of central nervous system effects[4, 182-184]. The risk of drug-induced toxicity is increased when nevirapine and efavirenz are combined[185, 186]. This sub-section of the review focuses on these three common NNRTI related adverse toxicities.

1.2.5.1.2.i. Cutaneous reactions: Skin rashes manifests in the early weeks of treatment with either NNRTI but it appear to be more commonly associated with nevirapine affecting between 4% to 38% of patients[4, 21, 22] compared with efavirenz at a slightly lower frequency of approximately 4.6% to 20%[4, 22, 23]. Postulations for the pathophysiology of these cutaneous reactions from limited human and animal data suggests that these rashes are probably cell-mediated hypersensitivity reactions[22, 187, 188]. The increased risk of rash in patients with higher CD4+ lymphocyte counts supports this postulation, particularly for nevirapine[189]. Risk factors for a greater risk of nevirapine-related rash include female sex, ethnicity (Hispanic, Chinese, and African), and individuals with earlier stages of HIV disease or a more profound initial increase in CD4+ lymphocyte count after initiation of treatment[21, 22, 190, 191]. Emerging evidence also suggests a genetic predisposition might exist for nevirapine hypersensitivity[192,193]. The risk factors for efavirenz-induced rash are less well described[21].

Clinically, rashes caused by NNRTIs are typically erythematous and maculopapular. Diffuse erythroderma, urticaria, erythema multiforme, blistering, desquamation, and mucosal involvement can occur. Life-threatening cases of Stevens-Johnson syndrome, toxic epidermal necrolysis, and erythema multiforme being reported in 0.1% of patients on efavirenz, compared with 0.3-1% reported with nevirapine[190, 194, 195]. The DRESS (Drug rash with eosinophilia and systemic symptoms) syndrome, often accompanied by fever and hepatitis, is well documented with nevirapine[196], but there is one reported case attributed to efavirenz[197].

There is evidence to support an initial 2-week lead-in period at half the recommended dose has been shown to reduce the risk of skin rashes with nevirapine by at least 50%[198,199]. Unfortunately, prophylactic use of corticosteroids or antihistamines to prevent hypersensitivity reactions due to nevirapine hypersensitivity reactions has not been shown to be of benefit, and indeed there is evidence to suggest that these interventions could in fact, increased risk of developing rash.[200]

1.2.5.1.2.ii. <u>Neuro-psychiatric toxicity</u>: Central nervous system (CNS) or neuropsychiatric disturbances have been reported in ~25%-70% of patients receiving efavirenz.[23,201-204] Symptoms include dizziness, headaches, confusion, impaired concentration, agitation, amnesia, psychotic symptoms, sleep abnormalities, abnormal dreams and insomnia. These symptoms usually arise within the first few days of treatment and lead to early discontinuation of efavirenz in ~4% -10% of patients, although some investigators have reported higher discontinuation rates[205]. The prevalence of most neuropsychiatric symptoms declines within a few weeks if therapy is continued[23, 201 - 204, 206]. In a substudy of ACTG A5095 study, measures of neuropsychological performance revealed no significant difference between patients who did and did not receive efavirenz[207]. While efavirenz recipients experienced more neurological symptoms at week 1 ($p<0.001$), this was not the case at week 4, 12 or 24.

It has however been noted in a minority of patients that, neuropsychiatric disturbances persist for several months or longer,[205, 206] or appear for the first time after several months of treatment with efavirenz[208]. Importantly, neuropsychiatric side-effects are an important risk factor for failure of therapy and for ‘blips’ in the HIV RNA level[208]. And although the mechanism of neuropsychiatric disturbances is not fully understood, they may be partly related to previous psychiatric disturbances or to neuropathic effects of HIV itself[205]. Studies in animals have suggested that the effects of efavirenz on cytokines may play a role in depression associated with efavirenz[206]. Sleep disturbances may play a role in the development of neuropsychiatric symptoms[205]. Neuropsychiatric disturbances appear to be more common in African Americans patients than in European American or Hispanic patients. This may be a consequence of a higher prevalence of the CYP2B6 T/T genotype, resulting in slower metabolism of efavirenz and higher plasma exposure[209]. Other studies have also given some (but not conclusive) evidence that a higher plasma level of efavirenz increases the risk of these problems[203, 205, 206]. Plasma monitoring may be considered in patients with persistent symptoms. Nevirapine does not appear to be associated with a high level of neuropsychiatric events and is considered in patients at a high risk of these symptoms[4, 203].

1.2.5.1.2.iii. Hepatotoxicity: Both efavirenz and nevirapine have been associated with hepatotoxicity, which may result in fulminant hepatitis and death[10, 25]. Hepatotoxicity occurs more frequently with nevirapine (1.4% to 17% of patients) than with efavirenz (1.1% to 8%)[24-28]. Most nevirapine hepatotoxicity is of early onset occurring within 12 weeks of initiating therapy[10]. The early onset and association with rash, fever and other constitutional symptoms suggests a probable immune-mediated mechanism for nevirapine hepatotoxicity[10,26]. There are reports from studies conducted in populations with a high prevalence of hepatitis B or hepatitis C co-infection documenting a later onset NNRTI hepatotoxicity, which is thought to be dose-related[24, 28, 210, 211]. In addition nevirapine-associated hepatotoxicity is associated with female gender, a low body mass index, and a high CD4 counts[10, 26, 190]. Evidence from the 2NN study suggested that high plasma trough concentrations of efavirenz, but not nevirapine, were associated with a higher risk of hepatotoxicity[212]. In a smaller cohort study, there was a correlation between higher plasma trough concentrations of nevirapine and hepatotoxicity, mainly in those co-infected with hepatitis C[210]. The cause-effect relation between high drug plasma concentrations and the risk of hepatotoxicity in patients with liver disease is controversial, since the high levels may correlate with more severe liver disease rather than reflect a dose-related toxicity[213]. Pharmacogenetic differences between populations that result in reduced NNRTI clearance could also account in part for the differences in the risk of hepatotoxicity observed in the different studies[214], if the mechanism is dose-related.

1.2.5.1.2.iv. Cross-reactivity between nevirapine and efavirenz: The WHO recommends substitution with efavirenz if nevirapine has to be discontinued because of cutaneous hypersensitivity (provided this was not life-threatening) or hepatotoxicity[181]. However, the extent of cross-reactivity between efavirenz and nevirapine is not clear. The molecular structures of efavirenz and nevirapine are very different suggesting that cross-reactivity may be less likely. A review of published literature from mainly retrospective studies, many of which were small, seems to suggest that there is cross-reactivity between efavirenz and nevirapine for cutaneous hypersensitivity[215]. The authors reported that recurrent reactions occurred in 30 (12.6%) of 239 reported patients with rash who were switched from nevirapine to efavirenz, compared with 8 (50%) of 16 patients who switched from efavirenz to nevirapine. Also, hepatitis did not recur in either the 11 reported patients who switched from nevirapine to efavirenz, or in the one patient who switched from efavirenz to nevirapine[215].

1.2.5.2 comparison of efavirenz with protease inhibitors

In combination with a backbone of two NRTIs, efavirenz has been shown to be superior to unboosted Indinavir[204, 216], more effective than unboosted nelfinavir[217, 218] and as effective as unboosted atazanavir[219]. The landmark ACTG A5142 study was a randomised, open-label, 96-week study of efavirenz versus ritonavir-boosted lopinavir- each administered with lamivudine plus zidovudine, stavudine or tenofovir- and efavirenz plus boosted lopinavir (an NRTI-sparing regimen). The primary endpoint analysis was the time to virological failure, defined as a lack of VL suppression by 1 log_{10} HIV RNA copies/ml or rebound before week 32, or a lack of VL suppression to <200 copies/ml or rebound after week 32. The group on efavirenz demonstrated a significantly longer time to this endpoint with a relative hazard ratio (HR) of 0.63 (95% confidence interval (CI) 0.45-0.87, P=0.006) [220]. The time to regimen failure (defined as virological failure or toxicity-related discontinuation of any component of the randomised regimen) also showed a benefit for efavirenz over boosted lopinavir (0.75; 95% CI 0.57-0.98; P=0.03), although this failed to reach the significance threshold adjusted for multiple comparisons (p=0.014). At 96 weeks, recurrent or new AIDS-defining conditions occurred in 4% of patients receiving efavirenz-based therapy versus 6% of those in the other arms. Immunologically, the efavirenz arm had the smallest median increase in CD4 count and virologically, significantly more patients treated with efavirenz-based therapy achieved a VL of <200 copies/ml or <50 copies/ml at 96 weeks than did boosted lopinavir-treated patients.

1.2.5.3 comparison of efavirenz with triple NRTIs

The ACTG A5095 double-blind randomised trial compared the use of the triple NRTI combination of zidovudine plus lamivudine and abacavir with efavirenz plus two or three NRTIs. This study was halted when an interim analysis at 32 weeks revealed that virological failure had occurred in almost twice as many of the patients treated with the triple NRTI regimen (21%) as in those on efavirenz plus either two or three NRTIs (11%; $p<0.0001$) [221]. Efavirenz-based therapy maintained high levels of efficacy over 3 years, with Abacavir adding no further benefit over efavirenz plus lamivudine and zidovudine[222, 223].

1.2.5.4 Comparisons of efavirenz with novel classes of antiretroviral agents.

[a] Integrase inhibitors: Raltegravir is an integrase inhibitor which has been compared with efavirenz in treatment-naïve patients in the 004 and STARTMRK studies. These two randomised controlled studies demonstrated comparable virologic efficacy of raltegravir to efavirenz, with CD4 increases and tolerability favouring raltegravir over efavirenz at the end of 96 weeks for 004 [224, 225] and 48 weeks for STARTMRK[226] respectively.

[b] CCR5 antagonists: Maraviroc is a CCR5 antagonist that inhibits virus/cell binding via inhibition of the co-receptor target CCR5 on the surface of host CD4 cells and thus demonstrates treatment effect in patients infected with the R5-using strain of the HIV-1 virus. The randomised, double-blind MERIT study compared the efficacy and tolerability of maraviroc (n=360) with efavirenz (n=361) in treatment-naïve patients

infected with R5 HIV-1, with both treatment groups also receiving combivir (zidovudine/lamivudine)[227]. At 48 weeks, maraviroc did show non-inferiority (margin 10%) compared with efavirenz for the primary endpoint of a VL<50 copies/mL (65.3% versus 69.3%; lower limit of one-sided 97% CI-10.9%). However, the mean change from baseline in CD4 cell count was greater for patients receiving maraviroc than in the efavirenz arm. Further analysis at 96-weeks confirmed the 48-week observations[228]. A Phase II dose-finding study of the CCR5 antagonist vicriviroc was discontinued because of a higher incidence of virological failure among patients randomised to vicriviroc 25mg or 50mg twice daily[229].

[c] novel NNRTIs: Rilpivirine (TMC278) and Etravirine (TMC125) are novel second generation NNRTIs. The Study of Etravirine Neuropsychiatric Symptoms versus Efavirenz (SENSE) trial was a phase 2 double-blind, randomised trial comparing etravirine with efavirenz in treatment naïve patients with the primary endpoint of neuropsychiatric events up to week 12 and HIV RNA suppression at week 48 as a secondary endpoint[230]. This study showed 400mg of etravarine and 2 NRTIs led to similar rates of HIV RNA suppression, compared with efavirenz with 2 NRTIs; 76% vs 74% respectively but 6.3% of patients on etravirine had on-going neuropsychiatric adverse events at week 48 visit compared with 21.5% for patients on efavirenz (p=0.011).

A dose-ranging study compared 25, 75 or 150mg of rilpivirine once daily with 600mg of efavirenz once daily (each added to two NRTIs) in 368 treatment-naïve patients. The primary end point was the proportion of patients with a VL of <50 copies/mL at 48 weeks, which was reached by 80%, 80% and 77% of patients treated with rilpivirine 25, 75 and 150mg, respectively versus 81% of those receiving efavirenz which were sustained at 96 weeks[231]. Both treatments were generally well tolerated; rash and nervous system disorders were less common with rilpivirine than with efavirenz. Rilpivirine continued to show sustained efficacy similar to efavirenz at week 192 with a generally more favourable safety profile[232].

In summary, there is at present no evidence that integrase inhibitors, CCR5 antagonists or novel NNRTIs are more effective than efavirenz in treatment-naïve patients, but further studies are in progress. These new agents are generally well tolerated and may have important roles after failure of initial therapy.

1.2.5.5. Efficacy of efavirenz in relation to HIV subtypes

NNRTIs are highly selective for HIV-1 and do not inhibit HIV-2. Efavirenz treatment has predominantly been studied in patients with HIV-1 subtype B, the most prevalent form in developed countries[233]. However, almost 90% of people infected with HIV worldwide do not subtype B virus[234]; globally 50% are infected with subtype C[235]. Studies have shown that subtypes B and C exhibit similar virological responses to efavirenz[234, 236, 237]. Soares et al[234] have reported that there is no difference in the accumulation of NNRTI resistance mutations between subtypes B and C.

1.2.5.6. Safety and tolerability of efavirenz

Efavirenz has been generally well tolerated in clinical trials. The risk of central nervous system toxicity on efavirenz has been review in sub-section 1.2.5.1.2.iv, however the use of efavirenz may be associated with the development of lipoatrophy and derangements of plasma lipids. The occurrence of lipoatrophy on efavirenz may result partly from effects on adipocytes including inhibition of lipogenesis and differentiation[238]. Lipodystrophy is more common when thymidine analogues, particularly stavudine, are included, in the NRTI backbone[239].

Efavirenz-containing regimens may modestly increase plasma lipid levels compared with a triple NRTI regimen[240]. The ACTG A5142 study showed no significant difference in the incidence of grade 3-4 elevations in low-density lipo-protein cholesterol with efavirenz versus boosted lopinavir[220]. However, grade 3-4 increases in triglyceride levels were significantly less common with efavirenz (2%) than with boosted lopinavir (6%; $p<0.05$) or efavirenz plus boosted lopinavir (14%; $p<0.05$). Overall, efavirenz appears to have generally neutral effects on lipids, but this is depends to a large extent on the accompanying NRTIs[239].

1.2.5.7. Use of efavirenz in special groups

1.2.5.7.i. Use of efavirenz in women of reproductive age

Recent statistics from the Antiretroviral Pregnancy Registry showed no increase in the risk of overall birth defects associated with drugs having sufficient reports of first-trimester exposure to detect at a 2-fold increase in risk[29]. There has been 18 reported birth defects out of 679 live births from first trimester exposures to efavirenz giving a prevalence of 2.7% (95% CI of 1.6% to 4.2%) which is a proportion deemed not substantially different than the CDC's birth defects of 2.72 per 100 live births identified among births from 1989 through 2003 [29]. Despite these observations, "efavirenz should not be used in pregnant women unless the patient's clinical condition requires such treatment", and "women of child bearing potential should undergo pregnancy testing before initiation of efavirenz" [30].

1.2.5.7.ii. Hepatitis B/C co-infection

Patients with HIV co-infected with hepatitis B virus (HBV) or hepatits C virus (HCV) have a significantly worse prognosis than those infected with HIV alone, and guidelines recommend early treatment of both conditions[111, 241]. In patients with HBV co-infection the NRTI backbone should include tenofovir plus lamivudine or emtricitabine because these agents have activitiy against both HIV and HBV. All antiretroviral agents have potential for hepatotoxicity, which is potentiated in the presence of HBV/HCV co-infection. Increased hepatotoxicity, including elevated liver enzymes, has been seen in patients co-infected with HBV/HCV (particularly HCV) receiving efavirenz-based regimens[28, 242-244]. It has been postulated that HBV/HCV co-infection results in higher exposure to efavirenz, leading to the hepatic side-effects observed. However, recent studies in patients with HIV have found no significant differences in efavirenz plasma levels between those with and without HBV/HCV co-infection[245, 246]. Thus the cause of the increased risk of liver toxicity remains to be elucidated. Overall, the available evidence suggests that NNRTIs have an important role in the management of HBV/HCV co-infected patients, with vigilant monitoring of hepatic function. The data are insufficient at this stage to indicate whether efavirenz or nevirapine should be preferred.

1.2.5.8. Effect of adherence on efavirenz on risk of virologic resistance

Adherence is undoubtedly a major determinant of virological failure and may lead to the emergence of resistance in patients with HIV, but the relationship between resistance development and adherence differ between antiretroviral drug classes[247, 248]. This is illustrated in a study involving 1191 patients initiating cART where it was found that those with <95% adherence to NNRTIs were significantly more likely to accumulate resistance mutations than those with ≥95% adherence (HR 7.0; 95%CI 3.4-14.5; p=0.0001), while adherence rates had little effect on resistance for PIs and NRTIs[249]. The proposed explanations for these observed class differences in risk for resistance selection, virological suppression and adherence are; differences in the relative fitness of resistant viruses (versus susceptible strains) in the presence of the drugs[250] as well as the pharmacokinetic and pharmacodynamic differences between the classes[251].

1.2.5.9. Quality of life on efavirenz

The use of efavirenz has been shown to improve health-related quality of life in the 2NN sub-study[252] and the INITO trial[253] where efavirenz-based cART was compared with nelfinavir-based cART. Neuropsychiatric symptoms with efavirenz impair quality of life in some patients, especially at the start of therapy[203, 205, 206]. In turn lower quality of life during treatment with efavirenz is a predictor of virological failure[254]. However, one report suggests that if patients are able to continue long-term efavirenz-based therapy, their quality of life can be good despite persisting symptoms [255].

1.2.6 Monitoring antiretroviral effectiveness in resource-limited settings

Access to antiretroviral therapy (ART) in Africa has increased remarkably over the past decade, beginning with a few thousand people and reaching five million people by mid-2010 [1]. This advance was because of the reduced cost of drugs, increased resources, expanded HIV testing, and activism. Other obstacles continue to limit the number of people taking ART and the ability of health systems to effectively monitor patients, including inadequate number of physicians and allied health staff[256] and limited laboratory capacity[257].

In many African countries the annual cost of quarterly CD4 cell counts and measurements of viral load exceeds the cost of generic first line ART[258]. In addition, establishing sophisticated laboratory services at relatively poorly equipped health facilities remains challenging. Consequently, many people taking ART in Africa receive either no routine laboratory follow-up or infrequent measurements of CD4 cell counts[257]. When CD4 testing is used as a routine component of care, most programmes offer it only every six or 12 months[259, 260] with a smaller proportion providing routine viral load testing[261, 262].

It is well known that high viral load and low CD4 cell count are independently associated with mortality[263-265], and changes in viral load and CD4 cell count during treatment have been associated with survival[265]. Routine monitoring of viral load and CD4 cell counts during ART, however, was adopted in well-resourced settings without studies indicating improved survival compared with careful clinical monitoring. One recent mathematical model showed little benefit and considerable cost even during 20 years of follow-up[266]. Furthermore, programmes in Haiti[267] and Malawi[268] have reported treatment success with clinical monitoring alone, although no groups with laboratory monitoring were available for comparison. By reducing or eliminating frequent laboratory monitoring, there is potential for increasing the number of people who could be treated. Reduced laboratory monitoring, however, might lead to premature or delayed changes to second line therapy, more antiretroviral resistance, or increased morbidity. Changes in CD4 cell counts do not accurately predict suppression of viral load[269-271]. A recently reported randomised clinical trial examined different monitoring strategies for individuals receiving ART found only marginal clinical benefits in terms of mortality associated with providing six monthly monitoring of CD4 cell count in addition to clinical monitoring in Uganda and Zimbabwe[272]. The authors of the DART study concluded that the addition of monitoring CD4 cell counts was not cost effective according to current WHO guidelines. Subsequently, a randomised clinical trial among patients receiving ART for HIV infection in Uganda found that routine laboratory monitoring using CD4 counts was associated with improved health and survival compared with clinical monitoring alone[273].

1.2.7. Pharmacology of efavirenz

The focus of this section of literature review is to highlight the impact of single nucleotide polymorphisms (SNPs) of hepatic enzymes involved in the metabolism of efavirenz on efavirenz exposure with particular emphasis on cytochrome P450 2B6 the major enzyme responsible for the phase I metabolism of efavirenz. This section begins with basic information on the pharmacokinetics of efavirenz, introduces the concept of pharmacogenomics of antiretrovirals, examines factors responsible for the modulation of the expression and function of CYP2B6, and concludes on the impact of potential and known pharmacokinetic interactions between antimalarials and efavirenz. This is because two chapters of this thesis are devoted to the pharmacology of efavirenz among Ghanaian HIV-infected patients. Chapter 8 explores the impact of single nucleotide polymorphisms in hepatic enzymes- cytochrome P450 2B6, 2A6 and UGT2B7 as well as the constitutive androstane receptor; a regulator of CYP2B6 induction- on mid-dose efavirenz exposure while chapter 7 examines the pharmacokinetic interactions between efavirenz and the antimalarial, artesunate which is a component of the recommended antimalarial combination therapy.

1.2.7.1. Pharmacokinetics of efavirenz

1.2.7.1.i. Absorption

Peak efavirenz plasma concentrations are reached by 5 hours following single oral doses in uninfected volunteers[30]. The time to peak plasma concentrations is ~ 3 to 5 hours and steady-state plasma concentrations of efavirenz are reached in 6 to 7 days[30]. The bioavailability of a single 600mg dose of efavirenz hard capsules in uninfected volunteers is increased by 17% to 22% by food[30]. Efavirenz is highly bound (~ 99.5% to 99.75%) to human plasma proteins, predominantly albumin[30, 274].

1.2.7.1.ii. Biotransformation

Efavirenz is oxidised to inactive 8-hydroxy, 8,14-dihydroxy, and 7-hydroxy efavirenz metabolites by the cytochrome P450 system predominantly by hepatic CYP2B6 with minor contributions from CYP3A4/5 and CYP2A6 [275, 276]. Efavirenz and its 3 hydroxy-metabolites undergoes conjugation to form N-glucuronides with UDP-glucuronosyltransferase (UGT) 2B7 as the main UGT isoform responsible for glucuronidation of efavirenz[276-279]. The CYP2B6 gene is highly polymorphic and is subject of considerable inter-individual variability in expression and function[280]. Also polymorphisms in CYP2A6 [281, 282] and UGT2B7 [283] have been shown to influence efavirenz exposure and will be subsequently reviewed.

1.2.7.1.iii. Elimination

Efavirenz has a terminal half-life of at least 52 hours after single doses and 40 to 55 hours after multiple doses[30]. Approximately 14% to 34% of a radio-labelled dose of efavirenz is recovered in the urine and <1% of the dose is excreted in urine unchanged[30]. The long half-life of efavirenz makes it suitable for once-daily dosing. The recommended dosage in adults is 600mg once daily. Genotypic testing for variants of the CYP2B6 allele could detect individuals at increased risk of neuropsychiatric adverse events but this is not routine practise. There is also no recommendation to adjust the dose of efavirenz according to race or sex.

1.2.7.2. Pharmcogenomics of antiretroviral medications

Cohort studies from the USA and Europe suggest that up to 50% of patients may modify their regimen and 25% may discontinue therapy, the majority for reasons of drug toxicity but also a significant number developing virological failure[284 - 288]. Both treatment failure and toxicity are costly: drug toxicity carries significant morbidity and salvage regimens are associated with higher pill burden and risk of adverse reactions, and drug treatment becomes more expensive with each successive failure. Strategies to make HIV treatment safer and more effective are urgently required. More than 20 approved antiretroviral (ARV) drugs from 6 mechanistic classes- nucleoside/-tide reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase strand transfer inhibitors (INSTIs), CCR5 antagonists and fusion inhibitors (FIs) are available for the treatment of HIV infection. Combination anti-retroviral therapy (ART) comprises three or more drugs from any of these classes. Following standard doses of anti-retroviral drugs, huge inter-individual variability has been observed, up to (coefficient of variation %) of 75% to >110%

occurs for NNRTIs and PIs, from prospective clinical trials and surveys of the Liverpool HIV Therapeutic Drug Monitoring (TDM) Registry[289]. The profound variability in exposure to antiretroviral drugs has important clinical implications and on the basis of the known plasma concentration-therapeutic response and concentration-toxicity correlation for NNRTIs and PIs, a therapeutic drug monitoring (TDM) has been proposed to optimize the exposure to these agents[290].

The causes of this variability are multifactorial and include poor adherence, body weight, gender and interacting medications[291]. Host genetic polymorphisms may account for some of the variation in pharmacokinetics and responses to ART. Ethnic differences in drug exposure are recognised: for example, drug exposure (AUC, Cmin) to indinavir (IDV) and saquinavir (SQV) is significantly increased in Thai patients compared to Caucasians[292]. There is also an observed longer elimination half-life of the NNRTI efavirenz (EFV) in African women discontinuing therapy compared with Caucasians[293]. In the 2NN study patients from Thailand and S Africa had lower clearances of EFV[294] than those from Europe or the Americas.

Genetic variability in the drug metabolising enzymes such as cytochrome P450, glucoronyl transferase) or drug transporters (e.g. MDR1, MRP1 & 2), prevalent at differing frequencies across ethnic groups probably explain some of the differences between populations, although other factors such as body weight may also contribute. For instance, considerable polymorphisms exist for cytochrome P450 2B6 [280] the major enzyme for detoxification of the NNRTIs efavirenz and nevirapine. In addition genetic variability exists for other cytochrome P450 enzymes involved in the metabolism of HIV drugs. Indinavir for instance is metabolized by the isoenzyme CYP3A5, which shows high genetic variability[295]. CYP3A5 expression is detectable in only 10-20% of Caucasians, 33% of Japanese and 55% of African-Americans[296]. The primary mutation responsible for lowered CYP3A5 expression (CYP3A5*3) results in erroneous mRNA splicing and reduced translation of functional protein. The presence of this variant has been associated with low or null expression of the CYP3A5 protein and consequently lead to a lower clearance of Indinavir with the attendant increased associated risk of renal calculi formation under Indinavir therapy[297]. Along the glucuronidation axis, mutations in UGT1A1 have been associated with hyperbilirubinaemia following therapy with indinavir[298] or atazanavir[299]. Whilst basal expression may be unaffected, the inducibility of these drug metabolising enzymes by rifampicin or other compounds may vary according to polymorphisms affecting gene regulation such as promoter variation or nuclear factors (PXR, CAR). These observations highlight the role of single nucleotide polymorphisms on the inter-individual variability of exposure to antiretroviral therapy. A thorough review of pharmacogenomics of all classes of antiretroviral therapy is beyond the scope of this literature review. However because the pharmacogenomics of efavirenz is of importance in this thesis, I will now focus the review on the highly polymorphic CYP2B6 and its correlates with efavirenz exposure.

1.2.7.3. The highly inducible and polymorphic cytochrome P450 2B6 gene and efavirenz exposure

It has been traditionally thought that CYP2B6 accounted for a minor proportion (<1%) of the total hepatic cytochrome P450 (CYP) content[300, 301] and played a negligible role in human drug metabolism in contrast to the predominant hepatic isoenzyme CYP3A4. However by utilising more sensitive and specific immunochemical detection methods subsequent evidence indicated that the average relative contribution of CYP2B6 to total hepatic CYP content ranges between 2% to 10% [302-306]. Furthermore it became obvious from these studies that the expression of CYP2B6 exhibited 20- to 250-fold inter-individual variability[280, 302-308]. It is conceivable that such inter-individual differences in hepatic CYP2B6 expression and enzyme activities may culminate in variable systemic exposure and therapeutic response to the growing list of drugs and chemicals known to be metabolised by this enzyme.

Elucidating the predominant mechanisms underlying the inter-individual variability in CYP2B6 expression has become the focus of several investigators and evidence is accumulating on plausible mechanisms such as polymorphisms in the CYP2B6 gene, transcriptional suppression by cytokines[309, 310], transcriptional activation by inducers[311, 312], enzyme inhibition, and allosteric activation[313]. There is a strong suspicion that genetic polymorphisms and/or differences in gene regulation may be the most significant contributors to the observed inter-individual variation in CYP2B6 expression in vivo.

1.2.7.3.i. Induction and Transcriptional regulation of CYP 2B6 Expression

The regulation of CYP2B6 transcription is recognised as one of the major contributors for the observed dramatic inter-individual variations in this isoenzyme. Mounting evidence reveals that the transcriptional regulation of this isoenzyme is intricate and multi-faceted. First, CYP2B6 shares mechanisms of transcriptional regulation with several other important drug metabolising genes such that a large number of CYP2B6 inducers have also been shown to induce CYP3A4, UGT1A1 (UDP-glucuronyltransferase 1A1) and several drug transporters such as the efflux drug transporter MDR 1 as well [314-317]. Second, among CYP2B6 inducers, some are also substrates for CYP2B6 and hence accelerate their own metabolism affecting clearance or toxicity. Such auto-induction of CYP2B6 has been observed with the non-nucleoside reverse transcriptase inhibitor efavirenz and the antimalarial artemisinin[315, 318-320].

Third, transcription of CYP2B6 is also regulated by delicate mechanisms at promoter sites by nuclear receptors. To date three promoter domains whose activation induces the transcription of CYP2B6 have been identified. These include a Phenobarbital-responsive enhancer module (PBREM) [321-323], a xenobiotic responsive enhancer module (XREM) [324] and more recently an okadaic acid responsive element [325]. Among the nuclear receptors involved in the regulation of CYP2B6 expression, two are crucial to the induction of the promoter sites and hence activation of the CYP2B6 gene. The first nuclear receptor to be identified in this context was the Constitutive Androstane Receptor [321] henceforth referred to as CAR and subsequently the Pregnane X Receptor[312, 326] also referred to as PXR. CAR has been shown to modulate CYP2B6 expression by binding to the nuclear receptor binding site 1 and 2- (NR1) and (NR2) sites within the PBREM domain and also to the XREM at its NR3 site [312]. PXR has also been demonstrated to bind to similar sites as CAR. Indeed both CAR and PXR bind to the NR1 domain of the PBREM with a stronger affinity than to the NR2 [312]. Secondly CAR and PXR bind to the NR1 and NR2 motifs of the PBREM and the NR3 motif of XREM as CAR-RXR and PXR-RXR heterodimers with RXR (Retinoid X Receptor) respectively.

There are however some differences in the interactions of CAR and PXR with the CYP 2B promoter sites with regards to the specificity of their ligand binding, the flexibility in their interaction with xenobiotics and their propensity to induce CYP3A4 alongside CYP2B6. For instance PXR ligands such as rifampicin and clotrimazole have been shown to induce CYP2B6 transcription along with CYP3A4 with little bias[312, 315, 327] whereas CAR specific ligands such as efavirenz, nevirapine, carbamazepine and phenytoin have a preference for induction of CYP2B6 over CYP3A4 [318, 328-330]. Furthermore CAR is less flexible in its interaction with xenobiotic ligands compared to the highly promiscuous PXR due to its smaller 675$Å^3$ ligand binding pocket as opposed to the larger 1290-1540 $Å^3$ binding pocket for PXR [331-334]. In spite of these differences accumulating evidence suggests that there is still a considerable substrate overlap between CAR and PXR and that the outcome of CYP2B induction is co-regulated by these 2 nuclear receptors. In summary, CYP2B6 gene expression is regulated by induction at proximal and distal promoter sites by the binding of heterodimers of either CAR-RXR or PXR-RXR. Efavirenz and nevirapine are specific ligands of human CAR and thence CYP2B6 inducers.

1.2.7.3.ii. CYP2B6 Gene Polymorphisms

Basis of genetic polymorphisms

Genetic polymorphisms arise as a result of an alteration in the nucleoside base sequence of the DNA composition of a gene. The most common genetic polymorphisms involve single nucleotide polymorphisms (SNP) where one base is substituted for another and is observed at a frequency of one SNP per every 1000 base pairs (bp) in the human genome. Polymorphisms may also arise from either an insertion or deletion of one, two or several base pairs (indels) or from gene duplication or from insertion or deletion of several copies of repeated base pair units called Variable Number Tandem Repeats (VNTRs).

By definition a single base change, occurring in a population at a frequency of more than 1% is termed a single nucleotide polymorphism (SNP). When a base change occurs at <1% of the population, it is termed a mutation. A transitional SNP involves the substitution of a purine such adenine (A) or guanine (G) for another purine or one pyrimidine such as cytosine (C) or thymidine (T) for another. A transversional SNP involves a change of a purine (A, G) for a pyrimidine (C,T) and vice versa. By nomenclature G>A means G which is the wild type has been substituted by the mutant A. G>A and C>T transitions account for approximately 25% of all SNPs in the human genome.

SNPs may affect the regulatory, the non-coding (intronic) or the coding (exonic) regions of a gene with differing resultant effects. A regulatory SNP may affect transcription factor binding and therefore affect gene expression. An intronic SNP may affect mRNA splicing and influence either the expression of the mRNA positively or negatively or affect mRNA activity as result of incorrect splicing. An exonic SNP may either alter amino acid structure of a protein referred to as a non-synonymous SNP or may not alter the amino acid structure called synonymous SNP. Synonymous SNP also called silent mutations may affect gene expression whiles non-synonymous SNP may affect activity via alteration of the tertiary structure of a protein or its post-translational modification.

CYP2B6 Single Nucleotide Polymorphisms

The genetic variations of CYP2B6 and its potential clinical significance have only been realised in recent years. Nevertheless, along with the realisation of the remarkable inter-individual variations in CYP2B6 expression and activity, the number of pharmacogenetic studies on this isoenzyme has been growing rapidly. To date, 28 characterised alleles, over 50 determined haplotypes and more than 100 described SNPs of the CYP2B6 gene have been documented and listed on the CYP allele nomenclature website (http://www.cypalleles.ki.se). Several genetic variations of CYP2B6 have been identified throughout its gene sequence including the 5´ promoter, introns and coding (exonic) regions. Functionally these genetic polymorphic alleles translate into a variety of phenotypic outcomes that include proteins with significantly reduced catalytic activity or a complete loss of CYP2B6 expression[280, 335].

CYP2B6 SNPs in coding regions

Lang and colleagues performed the first systematic analysis of the CYP2B6 genetic polymorphisms and focused on the nine exons in the coding region[280]. Their study led to the identification of nine base pair single mutations, of which five were non-synonymous amino acid alterations and 4 silent mutations. These SNPs alone or in combination resulted in six different CYP2B6 alleles designated as CYP2B6*2 (64C>T), CYP2B6*3(777C>A), CYP2B6*4 (785A>G), CYP2B6*5 (1459C>T), CYP2B6*6 (516G>T and 785A>G) and CYP2B6*7 (516G>T, 785A>G and 1459C>T). Among these alleles, CYP2B6*6, characterised by the combination of 516G>T in exon 4 and 785A>G in exon 5, was detected in about 15-40% of Asians and over 50% of African-Americans [280, 336, 337]. Further analysis of human liver mRNA discovered that aberrant splicing of the CYP2B6 gene resulted in the 2B6*6 allele which lacks exons 4, 5 and 6 and consequently displays a reduced function of mRNA and protein[338]. Phenotypically both heterozygous and homozygous carriers of CYP2B6*6 alleles exhibit a profoundly lower catalytic activity and a remarkably decreased protein expression, compared to the wild-type CYP2B6*1 allele carriers. Indeed CYP2B6*6 has been associated with aberrant efavirenz metabolism resulting in significantly supra-therapeutic plasma concentrations with its attendant therapeutic toxicities. The allelic variant (G516T) which is more common in African Americans (TT 20%) than Hispanics (6.7%) or Caucasians (3.4%) was associated with slower clearance of EFV leading to a hierarchy of EFV exposure (and associated CNS toxicity) in the rank order: African Americans > Hispanics > Caucasians[209]. Through the dedicated works of several independent research groups 19 more alleles in the coding region of CYP2B6 have been defined [301,308, 339]. Novel alleles which have also been characterised include CYP2B6*16 (785A>G and 983T>C), *18(983T>C), *27(593T>C) and *28(1132C>T) as well as four phenotypic null alleles *8 (415A>G), *11 (136A>G), *12 (296G>A), and *15 (1172T>A) http://www.cypalleles.ki.se/cyp2b6.htm. Although all these alleles are associated with amino acid changes, the functional consequences of each on CYP2B6 expression or function have varied extensively.

Among Ghanaians a number of mutations, particularly the G516T and T983C in CYP2B6, have been found to be relatively common, [35, 36] with the latter mutation having a gene frequency of around 7.3%. Whilst considerable work has been done showing the effect of the G516T mutation on efavirenz levels, less is known about the effect of the T983C mutation on either efavirenz or nevirapine levels. There is a strong suspicion that homozygotes for this mutation effectively have a null allele, since 2 homozygotes have been identified with severe efavirenz toxicity and estimated half lives of efavirenz over 3 months. It therefore seems likely that heterozygotes may have an intermediate phenotype with reduced metabolism of drugs such as efavirenz. The potential clinical implications for such patients are that patients with mutations such as the CYP2B6 G516T or T983C may develop toxicity to efavirenz more frequently, or be more likely to develop NNRTI resistance after stopping this drug, as has been suggested for the T983C mutation[340]. However few adequately powered studies have been conducted in Sub-Saharan Africa to investigate the role of SNPs in the CYP2B6 G516T/T983C composite genes and its impact on efavirenz pharmacokinetics and clinically relevant pharmacodynamic outcomes. Certainly in populations where there is a high frequency of mutant variants of clinically relevant SNPs in the CYP2B6 gene, the accessory/minor pathways for efavirenz namely the CYP2A6 and UGT2B7 may assume importance in clearance of this NNRTI, thus SNPs in the genes of these other enzymes could be additional sources for the wide inter-individual variability in mid-dose efavirenz exposure. Furthermore, given the high frequency of use of antimalarial drugs such as artesunate in this population, which are also metabolised by CYP2B6, there may be may be significant drug interactions and toxicity from such agents.

1.2.8. Interactions between antimalarials and antiretroviral medications

Both malaria and HIV/AIDS are important public health challenges in Sub-Saharan Africa because of their individual and combined impact on morbidity and mortality[341]. According to WHO recommendations, the first-line treatment for malaria in areas of endemicity is an artemisinin-based combination therapy containing a combination of either artesunate plus (amodiaquine, mefloquine, or sulfadoxine-pyrimethamine), artemether plus lumefantrine, or dihydroartemisinin-piperaquine[342]. Most HIV-infected patients in these malaria endemic regions receive a first-line antiretroviral regimen containing efavirenz[112]. Khoo S et al[343] and Skinner-Adams TS et al[344] have reviewed the pharmacological interactions between antiretroviral and antimalarial drugs in detail but the focus of this review is on the predicted and known interactions between artemisinin-based antimalarials and efavirenz of which few studies have explored the subject.

Among healthy volunteers, a 3-day amodiaquine-artesunate regimen was accompanied by increases in transaminase concentrations in two of five subjects receiving efavirenz, several weeks after completion of antimalarials[345]. The authors of this study[345] observed large increases in the amodiaquine area under the plasma concentration-time curve, maximum plasma concentration, and half-life. Hepatotoxicity of amodiaquine has been ascribed to the amodiaquine-quinoneimine metabolite[346], the extent of which may be increased by CYP3A4 induction. Given that efavirenz is known to induce CYP3A4, it has been inferred that the transaminitis observed and subsequent discontinuation of the study by German and co-workers[345] might have been due to increased production this hepatotoxic metabolite during efavirenz CYP3A4 induction. Furthermore, two cases of fulminant hepatitis during a short curative treatment with artesunate-amodiaquine in HIV-uninfected patients have been reported[347]. Indeed some authors on the basis of this study have contraindicated amodiaquine in patients receiving efavirenz[348] but the latest WHO guidelines on the treatment of malaria do not recommend any specific antimalarial treatment in HIV-infected patients[349]. It is noteworthy that the activation of artemisinins requires CYP3A4 thus the effect of administration of artesunate plus amodiaquine in patients receiving an efavirenz-based regimen may be difficult to analyse. Further studies are thus needed to evaluate these interactions separately, particularly the safety and tolerability of artemisinins used in HIV patients taking efavirenz because interactions leading to sub-therapeutic exposure of artemisinins could compromise the efficacy of this class of antimalarials in HIV-infected patients and potentially engender to emergence of artemisinin resistance. Conversely, if the interaction between artesunate and efavirenz leads to supra-therapeutic exposures to artemisinins the potential exists for toxicity to this antimalarial.

CHAPTER TWO

Methodology for chapters three to eight

2.0 Introduction

It became apparent during the writing up of this dissertation that the methods sections of the main chapters were lengthy in some chapters, themes of were overlapping in others and common statistical methods were employed. Thus it was thought that to make the transition from the introduction section of chapters to the results sections easier to follow, this chapter be devoted to describing methods used in the main chapters from three to eight. The sub-sections in this chapter are given numerical annotations in reference to the chapters they are used to hopefully make referencing easier.

2.1 Ethics approvals*:* Ethical permission for this study was given by the Committee on Human Research Publications and Ethics of the Kwame Nkrumah University of Science and Technology and the Komfo Anokye Teaching Hospital, Kumasi, Ghana (appendix 1).

2.2 Study settings and ART program in Ghana: All the studies conducted and compiled in this dissertation were performed at the HIV clinic at the Komfo Anokye Teaching Hospital. In January 2004, the HIV Clinic at the Komfo Anokye Teaching Hospital in Kumasi called “Chest Clinic” to avoid stigma was set up by the National AIDS Programme (NACP) to provide clinical care for patients living with HIV/ AIDS. Support for the clinic was provided by the Global Health fund for infectious diseases and Family Health International through the NACP. The Ghana Ministry of Health provided support for training of clinical and para-medical staff and anti-retroviral drugs through its various donor agencies.

At its inception the Chest Clinic in Kumasi was one of two Government approved centres for care of HIV patients. Patients with documentation of HIV infection were accepted for evaluation at the Chest Clinic as referrals from any public or private source within the Ashanti region and 6 other neighbouring regions due to the central location of Kumasi in Ghana. Other HIV seropositive cases were also referred to the clinic from voluntary counselling and testing unit situated at the Komfo Anokye Teaching Hospital. The vast majority of patients (>99%) evaluated were Ghanaians, but the programme is open to all ethnicities and to citizens from other countries. Clinical care of patients was provided by doctors, comprising of physician specialists, medical residents and house officers, nurses, public health nurses, pharmacists and dispensing technicians. Trained laboratory technologists were also provided to perform HIV serological screening and confirmation and CD4 T-cell enumeration.

To ensure a standard and complete approach to evaluating patients, a case record book was created where demographic data, clinical and laboratory information, treatments administered and clinical events were recorded. Initial evaluation of patients were often performed by trained and experienced nurses and includes medical history, physical examination, CD4 cell count and HIV sero-type 1 or 2. The objective of this initial assessment is to screen for the presence or otherwise of opportunistic infections and malignancies and eligibility for anti-retroviral therapy as well as prophylaxis with Co-trimoxazole according to Ghanaian national guidelines. In the first months of the programme, ART was initiated for individuals with either CD4 cell count of less than

200/μl or those in World Health Organisation (WHO) stage III or IV. In 2007 however these guidelines were revised to initiate ART for HIV patients with CD4 cell counts below 350/μl regardless of (WHO) clinical stage. All patients, whether starting ART or not were prescribed multivitamins. Patients not meeting eligibility criteria for ART were monitored six monthly by CD4 counts and clinical examination for new opportunistic infections. For those eligible for ART, further laboratory tests assessing haemoglobin concentration, serum urea and creatinine, serum alanine transaminase (ALT), serum aspartate transaminase (AST) and recently fasting serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglyceride concentrations were then performed to guide ART selection. Patients who were eligible for ART must have disclosed their serostatus to an adherence monitor who would supervise ART intake and help bring the patient to the clinic in the event of any serious adverse events, new opportunistic infections or malignancies and report any mortality to the clinic.

First-line ART regimens were prescribed by doctors and comprised of lamivudine (3TC) plus either zidovudine (AZT) or stavudine (d4T) plus either nevirapine (NVP) or efavirenz (EFV). The choice between the use of either AZT or d4T is determined primarily by availability but AZT is avoided in anaemic patients with haemoglobin concentration below 10g/dl. Efavirenz was avoided in females in their reproductive age due to reported teratogenicity. ART initiation was deferred in patients on intensive phase of anti-tuberculous therapy and started during the continuation phase with an efavirenz based ART to avoid NVP-rifampicin interactions. For patients with CD4 cell counts less than 200/μl or in WHO stage III or IV, co-trimoxazole was administered.

Patients who were found to have either HIV-1/ -2 dual or HIV-2 mono-infection were initiated on 2 NRTI's and a protease inhibitor of either nelfinavir or ritonavir boosted lopinavir.

The initial follow-up schedule for those starting cART includes 4 visits during the first 3 months, with special focus on adherence and detection of adverse events. During these first 3 months, haemoglobin concentration, serum ALT and AST are determined at week 2, 4, 8 and or 12 weeks post-initiation to detect incident or worsening anemia especially in patients on AZT-containing regimen, skin rash and hepatotoxicity in those on either NVP or EFV-containing ART. Subsequently follow up occurs every 3 months with CD4 cell counts, haemoglobin concentrations, ALT and AST performed every 6 months unless there is a clinical indication to otherwise perform more frequent laboratory monitoring. Those patients receiving cART present monthly to the clinic to collect their antiretroviral medications. Each dispensation includes a 2- to 3-day buffer of extra pills. Pre-registered family members or other treatment partners, also called "monitors", were permitted to collect a patient's medication. Adherence counselling was performed by nurses and pharmacy technicians. Patients on ART who defaulted on scheduled visits for more than 2 weeks were traced up in their homes by community health workers and peer education officers. However, due to wide geographical distribution of cases, incorrect residential identification numbers and large enrolment numbers our ability to track all such patients were outstripped. Therefore, the clinic was not able to attempt home visits for all defaulting patients however those with access to mobile phone were contacted using telecommunication.

Because routine use of viral load testing was not a part of the Ghanaian national guidelines, we relied principally on clinical and immunological estimates of treatment failure. Treatment failure is defined in our programme as (1) Clinical failure is the occurrence of a new opportunistic infection or malignancy signifying clinical disease progression, the recurrence of prior opportunistic infection or onset/recurrence of WHO stage 3 or 4 conditions and/or (2) Immunologic failure is the return of CD4 counts to pre-therapy baseline, or below and/or more than 50% fall from on-therapy CD4 peak-level (and/or more than 50% fall in CD4), or persistent low CD4 of less than 100 cells/µl after one year of therapy without other concomitant infection to explain the low CD4. Where clinical or immunological failure is suspected or confirmed, clinicians would request for a viral load test to be performed at the patients expense to inform a decision to switch to second line ART. However the high cost of viral load testing has prohibited its routine use in clinical decision-making. Until 2011, second line nucleoside/-tide reverse transcriptase options included tenofovir, abacavir and didanosine and protease inhibitors (PI) available were nelfinavir or ritonavir-boosted lopinavir.

Laboratory testing

A CD4 cell enumeration was performed at a central laboratory with a Beckman Coulter Epics XL-MCL 4-colour Flow Cytometer (Beckman Coulter, Inc, Miami, Fla). Complete blood counts with haemoglobin concentrations, white blood cells and platelets counts were done centrally using Sysmex XT-1800i automated haematology analyser (USA). A limited number of serum biochemical tests offered free of charge to the patients were performed namely alanine transaminase and aspartate transaminase to assess hepatic injury, serum creatinine concentrations for evaluating the renal function and fasting lipid profile were performed at the Chemical Department of KATH using the multi-parameter Roche Cobas Integra® 400 plus, Roche Diagnostics, Mannheim, Germany. Hepatitis B serology was performed to determine HBSAg seropositivity using the Determine kit routinely when kits were available at the department of serology at the Komfo Anokye Teaching Hospital. As part of another study called the HEPIK (HIV HBV Project in Kumasi) study, hepatitis B screening serology has been performed for a proportion of patients in the clinic.

Data collection and analysis

Since the inception of the clinic in 2004, nearly 11,000 HIV patients have enrolled to date, out of which about 5000 patients have been started on ART however no systematic data collection and analysis has been conducted to date. Thus, detailed information on effectiveness of cART, the proportion and reasons for loss to follow-up (LTFU), and incidence and impact of TB and other opportunistic infections, are widely lacking. Although the public health unit of the Komfo Anokye Teaching Hospital collects data on patients registered in the clinic and follow up clinical and laboratory data, initial attempts to use that database proved problematic since collection of data were not structured and data was not of sufficient quality.

To standardise data collection for the present analysis, I designed a data collection proforma (see appendix 2) for this purpose. Information on patient registration, initial clinical assessment for antiretroviral medication eligibility, follow-up visits and laboratory data recorded in patient folders (see appendix 3) were extracted on to the data collection proforma. Patients were included in analysis only when there was evidence in their folders that ART was initiated from January 2004 to December 31st 2010. Patients initiating therapy within 2011 were excluded from the present analysis because the primary objective of the present analysis was to determine the long-term effectiveness of first-line ART which required at least one year of follow-up. Each folder was examined by FSS for initiation of ART and data collected unto this proforma under the supervision of ROP who served as the local supervisor. Baseline data collected included age, gender, residential address, WHO clinical stage and the HIV-associated co-morbidities for the WHO clinical stage, weight and height for body mass index calculation, hepatitis B sero-status and HIV sero-type if known together with baseline CD4 count at initiation often done within the last 3 months before therapy initiation, haemoglobin concentration, ALT and AST as well as creatinine and fasting lipid profile if available. During therapy, CD4 counts, AST, ALT, haemoglobin and seldomly serum creatinine concentrations were recorded along with body weight changes and any documented ART-related toxicity, AIDS-defining and non-AIDS-defining events as well as clinically and immunologically defined treatment failure, drug substitutions and switches to second line cART. After data had been collected on the proforma, they were given to a data entry clerk to enter data into an excel spread sheet. Validity of data recorded from folders onto proformas and subsequently onto the excel spread sheet was assessed by ROP who randomly took 10 folders from every 100 folders assessed. When occasional inconsistencies were observed they were corrected upon consensus. Data collected in this manner were analysed to answer specific questions in chapters 3 to 7. Pregnant women initiating antiretroviral therapy were excluded from all analysis.

2.3. Methods for chapter three

The study presented in this chapter is cross-sectional and descriptive in design with the primary objective of presenting data on the baseline characteristics of patients initiating cART in this cohort and their vital status at closure of data for analysis. The severity of anaemia and baseline derangement in liver functions tests and renal impairment were assessed as follows:

Severity of anemia was classified as follows: severe anemia=haemoglobin <8 g/dl, moderate anemia= haemoglobin 8-10g/dl in females and 8-11g/dl in males, mild anaemia= haemoglobin 10-12 g/dl in females and 11-13 g/dl in males; none=haemoglobin >12g/dl in females and >13g/dl in males.

Severity of hepatic damage were graded based on the ALT level and defined in accordance with AIDS Clinical Trials Group criteria[350] in the following manner: grade 1, 1.25 – 2.5 times the upper limit of normal (X ULN); grade 2, 2.6-5.0 X ULN; grade 3, 5.1 – 10.0 X ULN; grade 4, >10 X ULN. For purposes of analysis, grade 1 and 2 hepatotoxicity was classified as mild whereas grades 3 and 4 hepatotoxicity were graded as severe.

Three formulae were used to calculate estimated glomerular filtration rate from serum creatinine concentrations namely the Cockroft-Gault[351] equation, the 4-variable Modified Diet in Renal Disease (MDRD)[352] equation and the Chronic Kidney Disease-Epidemiological Collaboration (CKD-EPI)[353] equation. The formulae used are presented below:

Cockroft-Gault formula:

eGFR= (140-age) x weight (in kilograms) x [constant]/serum creatinine in μmol/l

constant= 1.23 for men and 1.04 for women.

MDRD formula:

eGFR= 32788 x serum creatinine $^{-1.154}$ x Age$^{-0.203}$ x [1.212 if black] x [0.742 if female]

Where serum creatinine is in μmol/l.

CKD-EPI formula:

eGFR=141 x min (SCr/k, 1)^{a} x max (SCr/k, 1)$^{-1.209}$ x 1.018 if female x 1.159 if black.

SCr = serum in mg/dl,

k=0.7 for females and 0.9 for males,

a is -0.329 for females and -0.411 for males.

Min indicates the minimum of the SCr/k or 1, and max indicates the minimum of SCr/k or 1. After calculating the eGFR, correction for body surface area was then performed. The MDRD and CKD-EPI- derived eGFRs are expressed as ml/min/1.73 m^2 because the equations were derived by comparison with iothalamate-measured GFR, which itself is expressed as ml/min 1.73 m^2. However the eGFR derived by using the Cockroft-Gault equation was converted from ml/min to ml/min/1.73 m^2 by multiplying calculated values by 1.73, and dividing by body surface area (BSA). BSA was calculated using the following formula[354]:

Body surface area = 0.007184 x height$^{0.725}$ x weight$^{0.425}$

Stages of renal impairment were classified using the Kidney Disease Outcome Quality Initiative staging criteria[355] as shown in Table 2.1 below. Stages 1 and 2 are usually applied to patients with evidence of kidney disease (other than reduced GFR), such as proteinuria, haematuria and known kidney disease. However in this study urine analysis data was not available because it is not routinely performed in the initial assessment of patients in evaluating for eligibility for initiation of cART.

Table 2.1 Stages of chronic renal disease.

Stage	Clinical	GFR (ml/min)
1	Normal	>90
2	Mild impairment	60-89
3	Moderate impairment	30-59
4	Severe impairment	15-29
5	End-stage renal failure	<15

Hypocholesterolaemia were defined according to the US National Cholesterol Education Programme guidelines[356] as fasting cholesterol concentrations of < 150mg/dl while hypertriglyceridaemia was defined as fasting triglyceride concentration >200mg/dl.

At closure of data for analysis it was realised that nearly 24% of patients were lost to follow up. To gain an insight into their vital status and also the potential reasons for loss-to-follow up, a telephone survey was conducted among 210 patients or contacts who had given their telephone details primarily to assess the vital status of patients as dead or alive.

Statistical analysis (chapter 3)

Normality of distribution of continuous data was assessed using the Kolmogorov-Smirnov (KS) test. Univariate comparisons of continuous of data were performed using either the Mann-Whitney's U-test or the Wilcoxon rank sum test for data that did not follow a Gaussian distribution while Student's t-test was used to compare means of data of Gaussian distribution between two groups. For more than two groups, an analysis of variance (ANOVA) was performed for data with Normal distribution with Bonferroni's test to adjust for p-values to account for multiple comparisons while the Kruskal-Wallis test was used for comparison of data which did not follow a Normal distribution. Comparisons of dichotomous data were performed using the chi-squared test or Fisher's exact test and multiple logistic regression analysis was performed to identify factors associated with HIV-related renal impairment and elevated alanine transaminase among patients before initiating therapy. Kappa statistics was used to assess agreement between the 3 formulae used to calculate eGFR. In assessing the inter-rater agreement between the 3 formulae for stages of renal impairment using eGFR, the Cohen kappa statistic κ-value with the standard error as well as a linearly weighted Fleiss' kappa values were reported.

All univariate analyses were performed using GraphPad Prism version 4. Multivariable logistic regressions performed using SPSS version 17. For all analysis, a 2-sided p-value <0.05 was set as the level of statistical significance.

2.4. Methods for chapter four

The design of this study was retrospective, longitudinal and analytical among patients who started cART in the cohort. For this analysis, AIDS-defining event, non-AIDS defining clinical event, immune reconstitution inflammatory syndrome, loss-to-follow up, death and adherence to therapy were defined as follows:

An AIDS-defining clinical event was defined as the occurrence of any stage 3 or 4 opportunistic infectious or malignancy according the World Health Organisation (WHO)[112] criteria while patient was on cART. This staging system is based on clinical findings that guide the diagnosis, evaluation, and management of HIV/AIDS in resource-limited settings and it does not require a CD4 cell count. The list of AIDS-defining events under the WHO criteria is shown in Table 1.1 under section 1.2.1.6 in the literature review.

The diagnosis of a non-AIDS clinical event was defined using established Division of AIDS (DAIDS) tables for Grading Severity of Adult Adverse Experiences (NADEs). The conditions classified under NADEs included cerebrovascular accident (stroke), cerebral/sub-arachnoid haemorrhage, myocardial infarction, coronary artery disease, congestive cardiac failure, end-stage renal disease, renal failure, cirrhosis of the liver, esophageal varices, hepatic failure, hepatic coma, hepatic encephalopathy, intestinal adenocarcinoma/lymphoma, penile carcinoma, small cell lung carcinoma, malignant melanoma, hepatocellular carcinoma, squamous cell carcinoma, and squamous cell carcinoma of the anus[358, 359].

Other medical diagnoses that did not fulfil the criteria for AIDS-defining events or serious NADEs (as defined above) were recorded and presented as "medical co-morbidities on cART". These included conditions such as malaria, urinary tract infections, new onset diabetes mellitus or systemic arterial hypertension, and so forth.

Immune reconstitution inflammatory syndrome (IRIS) is defined as a paradoxical worsening of treated opportunistic infections or the unmasking of previously sub-clinical, untreated opportunistic infections or malignancies[360]. In the present cohort, IRIS was detected clinically in most instances and defined as the paradoxical worsening of a previously treated opportunistic infection for which the clinician was able to demonstrate radiological evidence of exacerbation in the cases of tuberculosis or cerebral toxoplasmosis, fundoscopic evidence of vitreous inflammation in the case of CMV retinitis. Cases of herpes zoster IRIS were defined clinically. Due to the inherent difficulty of differentiating an opportunistic infection with normal presentation and a disorder with presentation that is compatible with unmasking IRIS in our setting, the unmasking type is IRIS was not documented in charts in this cohort.

Loss to follow up was defined as missing a clinic appointment by at least 3 months of the last scheduled visit to clinic.

Death was defined as the demise of a patient from causes related to HIV/AIDS or from toxicity from antiretrovirals or other non-AIDS related causes if known. Confirmation of death were by death certification by medical doctors and verbal autopsy by patients.

Adherence in the clinic is routinely assessed using pill counts. Patients' adherence were therefore routinely classified as excellent at each clinic visit if adherence level of ≥95% was achieved. Poor adherence was defined as any documented evidence of <95% of adherence during follow up.

Statistical analysis (chapter 4)

Median of CD4 counts and body mass indices were compared longitudinally with baseline values in patients who remained on treatment using Mann-Whitney U-test. Crude incidence rate of events were calculated in person-years of follow-up with 95% confidence intervals calculated using Normal approximation to the Poisson distribution.

Risk factors associated with death, loss to follow-up, AIDS-defining events were assessed using multivariable Cox proportional hazards regression with factors attaining a significance level of <0.10 in univariate analyses included in the final model. For these survival analyses, the month in which patients started cART was set as month zero and one day of follow-up was added to patients who did not attend any follow-up visits after initiating therapy. Time to events of interest namely AIDS-defining events, loss-to-follow up or mortality was calculated by subtracting the date of the event from the date on which the patient was started on cART. Patients were censored at December 31, 2011 if there were no event of interest. Explanatory variables included in survival analyses were selected on the basis of their well-recognised impact on clinical outcomes such as AIDS-defining events, loss-to-follow up and deaths. The cumulative incidence of loss to follow up and deaths were calculated using the Kaplan Meier methodology. Adjusted analysis was performed to determine the independent effect of baseline renal impairment on the risk of death by a primary and sensitivity analysis. In primary analysis patients were right-censored as described above but in sensitivity analysis patients lost-to-follow up were assumed to have died (missing=failure). Risk factors associated with poor adherence were analysed using a multiple logistic regression analysis. For all analysis, a 2-sided p-value <0.05 was set as the level of statistical significance.

2.5. Methods for chapter five

Clinical and laboratory data recorded in patient folders were retrospectively collected using a data collection proforma (refer to appendix 2).

Data collection (description of criteria relevant to this chapter)

All patients were routinely screened clinically for toxicity on cART at each clinic visit in a standardised approach and recorded in case notes. Amongst the NNRTIs data was collected on skin rash, neuropsychiatric adverse events, and hepatotoxicity. Mitochondrial toxicity namely peripheral neuropathy and lipoatrophy are recorded as NRTI-related toxicity and anaemia was attributed to zidovudine unless there was another more probable cause.

Anaemia: To enable clinicians to detect anaemia due to toxicity or other causes, routine full blood counts were performed half-yearly or upon clinical suspicion of anaemia. Anaemia was defined as a haemoglobin concentration of ≤ 12g/dl for women and ≤ 14g/dl for men[350]. Anaemia was graded as severe or life-threatening if the haemoglobin concentration was ≤ 8g/dl. In patients who had any degree of anaemia before starting therapy, worsening anaemia was defined a haemoglobin concentration ≤ 8g/dl or if there were a decline by more than 2.5g/dl from baseline[350]. Zidovudine was implicated as the cause of anaemia for patients taking this medication unless another clinical explanation was recorded in the folder of patient.

Less common side effects of zidovudine such as hyperpigmentation and hypersalivation were also recorded.

Skin rash definitions: The types and severity of skin rash were commonly described by clinicians in patients' folders. Based on these descriptive accounts severity of skin rash was staged, using criteria published by from the 2NN sub-study[361] as Level I: erythema or hyperpigmented rash; level IIA: diffuse maculopapular rash; level IIB: urticaria; level III: rash plus constitutional symptoms such as fever, myalgia, pruritus and malaise or angiooedema, serum sickness-like reactions, Steven's Johnson syndrome; level IV: toxic epidermal necrolysis. A cross-reactivity NNRTI-cutaneous reaction was defined as the re-appearance of a rash attributable to NNRTI, after substituting one NNRTI for another[14]. When the physician did not attribute skin rash to drug toxicity, the cause of the skin rash was recorded but not included in the analysis for toxicity. FSS and ROP staged the severity of skin rash based on description of rash in the patient's folder when this was not clearly documented in the folder.

Definitions of hepatotoxicity grades: Hepatotoxicity grades were based on ALT level and defined in accordance with AIDS Clinical Trials Group criteria[350] in the following manner: grade 1, 1.25-2.5 times the upper limit of normal (x ULN); grade 2, 2.6-5.0 x ULN; grade 3, 5.1-10 x ULN; grade 4, >10 x ULN. In order to avoid selection bias favouring the inclusion of patients with very high baseline ALT levels, severe hepatotoxicity was defined as grade 3 or 4 increases in ALT level or an increase in ALT level of greater than 100 IU/l from baseline as previously described[362]. Furthermore, when a patient was noted to have had hepatic enzyme elevations, the reasons for those elevations as recorded by the attending clinicians were noted and if was thought not to be antiretroviral therapy related, that data was not included. Among factors that could cause hepatic enzyme elevations were heavy alcohol use which were screened for routinely during follow up.

Neuropsychiatric symptoms associated with use of NNRTI's especially those on efavirenz were routinely recorded without any standardised assessment of severity.

Individual neuropsychiatric symptoms recorded in patients' records for which the attending clinician attributed to NNRTI were noted.

Mitochondrial toxicity Mitochondrial toxicity data was collected with emphasis on the 4 common events namely peripheral neuropathy, lipoatrophy, symptomatic lactic acidosis and pancreatitis. Neuropathy was clinically assessed based on patients' symptoms and stavudine or zidovudine was substituted in severe cases upon the clinical judgement of HIV physician. Patients were assessed for lipoatrophy (loss of subcutaneous fat in the face, arms, legs, cheeks, buttocks) at every encounter by self-report and clinical assessment. Decisions to substitute stavudine were guided by the clinical severity of lipoatrophy based on the combined assessment of the clinician and the patient's preference and perception of the body changes. Lactic acidosis was diagnosed symptomatically since there were no facilities to routinely measure serum lactate concentrations. Clinical suspicions of pancreatitis were corroborated by performing serum amylase.

Outcome measures

The primary outcome was time to occurrence of a specific ART-associated toxicity.

Statistical analysis (chapter 5)

The cumulative incidence of each specific ART-associated toxicity was calculated using the Kaplan-Meier methodology and the median time to occurrence of the first event calculated using the Mann-Whitney's U-test, when the median time could not be determined by Kaplan-Meier methodology. Patients were censored either at the date of first ART-related toxicity, at the last visit for patients that died, were transferred out or were lost to follow-up and at December 31, 2011 for the remainder. Patients switched to second line ART regimens due to either clinical or immunological failure were censored at the date of switching and defined as having experienced no specific first line drug-related toxicity event. A risk factor analysis was performed using multivariate Cox proportional hazards regression model. Collinearity between variables was assessed. A backward selection method, retaining those variables with p-values <0.10 in the final model. In order to evaluate the associations between individual ART-associated toxicity and all-cause toxicity on the risk of poor adherence, a multiple logistic regression analysis was performed. Risk factors identified to be independently and significantly associated with the risk of poor adherence were adjusted for in the final logistic regression models to assess the independent effects of specific and all-cause ART-related toxicity on adherence. The level of significance was set at $p<0.05$.

2.6. Methods for chapter six

Inclusion criteria: Patients were included in this analysis if they started ART within 1st January 2004 to 31st December 2010. They should have been ART naïve, more than 15 years, and should have started either efavirenz or nevirapine with a backbone of either zidovudine plus lamivudine or stavudine plus lamivudine. Data were closed at 31st December, 2011.

Outcome measures: The primary outcome measure of treatment effectiveness for this comparison between EFV vs NVP-based cART was treatment failure defined as a composite of deaths, clinical disease progression and all-cause treatment discontinuation.

1. Clinical progression after initiating cART is the occurrence of a new opportunistic infection or malignancy, the recurrence of prior opportunistic infection or onset/recurrence of WHO stage 3 or 4 conditions.

2. All-cause treatment discontinuation includes treatment discontinuations due to drug toxicity, treatment failure necessitating change to second line therapy and other physician and patient preferences.

In secondary analysis, the outcome measures of interest were:

1. Changes in the CD4 + T-cell counts over time.

2. Changes in body mass index over time

3. The impact of the NRTI backbone on the primary and secondary outcome measures.

Statistical methods (chapter 6)

Differences in baseline characteristics such as age, CD4 cell counts, haemoglobin concentration and serum biochemistry results were compared using either the Mann-Whitney U-test for continuous variables that did not follow normal distribution or unpaired student's t-test for normally distributed continuous variables. Dichotomous or nominal variables such as gender, year of initiation of treatment as well as the cART regimens initiated were compared using the Chi-squared test.

In evaluating the primary outcome measure, two types of analyses were performed namely the Cox proportional hazards regression to model time to therapeutic failure and logistic regression analyses to model risk of therapeutic failure. Cox proportional hazards regression were used to model the individual and simultaneous effects of the initial NNRTI, baseline variables and pill count assessed adherence on time to the composite end point of deaths, clinical progression and NNRTI discontinuation. All available variables were included a priori in multivariate models and were stratified into discrete categories: sex (male vs female), age (≥ 40 years vs < 40 years), WHO clinical stage (3 and 4 vs 1 and 2), BMI (16kg/m^2 vs ≥16 kg/m^2), CD4 count (<200 cells/mm^3 vs ≥200 cells/mm^3), haemoglobin concentration (<8g/dl vs ≥8g/dl), calender year (2004-2006 vs 2007-2010), NRTI backbone (stavudine plus lamivudine vs zidovudine plus lamivudine), adherence (excellent vs poor) and NNRTI (efavirenz vs nevirapine). These variables were chosen because they were potential confounders of therapeutic failure.

Composite primary end-point analysis: In analysing the primary outcome measure of treatment failure defined as either death or clinical progression or discontinuation of NNRTI for any reason, time to the first occurrence of any of the three components of the co-primary outcome measure was calculated by subtracting the date of the event from the date of initiation of cART. Patients were censored if none of the events in the co-primary endpoint was not observed at the time of last visit for patients that were transferred out or were lost to follow-up and at 31st December, 2011 for the remainder. Treatment modifications in the NRTI backbone were disregarded. In both the Cox regression and logistic regression models, therapeutic failure was defined as (as defined above) i.e missing = censored for primary analysis. In sensitivity analysis patients lost to follow-up were considered to failed therapy (missing=failure). Interaction between covariates was tested by including multiplicative terms in regression models. For these analyses any factor that was significant at the 10% level in univariate analyses (p<0.1) was included in multivariate analysis. In multivariate analysis, statistical significance was attained if $p<0.05$. All p-values were exact and two-tailed.

Imputation of data for missing data for Cox and logistic regression analyses: Missing CD4 counts were imputed as below 200 cells/mm^3 (n=30; 24 for efavirenz and 6 for nevirapine), missing WHO clinical classification was imputed as having AIDS defining event at baseline (n=456; 269 for efavirenz vs 187 for nevirapine), missing BMI (n=100, 70 for efavirenz and 30 for nevirapine) for females and males were imputed with the average of the median BMI for both sexes respectively.

Changes in CD4 counts and BMI: Immunologic response measured by CD4+ T-cell count changes and anthropometric changes assessed using body mass index were compared by pair-wise comparisons of medians at 2months and subsequently 6 monthly intervals between the two groups. Where repeated comparisons are performed a p-value <0.01 was set as the level of significance to detect a difference between two groups. Changes in CD4 counts over time on cART were modelled with a generalised mixed effects linear model by using log link and Poisson distribution. The treatment (nevirapine and efavirenz), time since the initiation of therapy, backbone (AZT plus 3TC or d4T plus 3TC) and their interactions were specified as fixed effects in the model whilst the patients were specified random effects in order to account for the repeated observations per patient over time. Changes in BMI was modelled using a linear mixed effects model with identity link and Gaussian distribution. The model for change in BMI by NRTI backbone with NNRTI (nevirapine and efavirenz) and time since the initiation of therapy and their interactions specified as fixed effects and patients as random effects. Thus both the mean CD4 cell counts and BMI before therapy and the slope of change over follow-up time in the nevirapine and efavirenz groups could be compared by the Fisher's *F test*. BMI, gender and WHO clinical stages were included as explanatory variables in both models. Also, pre-therapy CD4 and pre-therapy BMI were included in the two models respectively.

2.7. Methods for chapter seven

The study was a prospective, open label, observational pharmacokinetics study including HIV-infected patients on efavirenz-based cART with control patients whose HIV status were unknown. The primary objective was to determine the effect of steady state therapy of efavirenz on artesunate/DHA pharmacokinetics. Secondary objectives were to investigate the effect of artesunate on steady state concentrations of efavirenz and to evaluate symptoms or laboratory features of toxicity of artesunate/DHA and efavirenz.

Sample size calculation

Basing the sample size on the primary comparison, namely artesunate PK in patients on steady state efavirenz, the sample size was calculated on the basis of a previous study of coartemether[363]. Using this data, assuming a first dose AUC (SD) of 204 (107) ng*h/mL and a last dose value of 63.6 (72.5) ng*h/mL, a total of 20 patients in each group will provide 83% power to detect a 50% difference in AUC for artesunate between each group.

Ethical Considerations

The main ethical issue in this study was the potential to recruit patients who are treated for malaria with what may be regarded as sub-optimal therapy, namely artesunate without an additional antimalarial drug, which was not in line with current WHO recommendations. On the other hand, a significant number of patients in Ghana (and other malaria-endemic countries) are treated with artesunate alone due to issues of affordability of combination therapy, drug supply and toxicity of some combination therapies. Patients who are treated with artesunate alone, were only approached regarding inclusion in the study if the patient has been informed that artesunate monotherapy is probably inferior to combination therapies, and the patient's physician has already decided to treat the patient with artesunate alone, rather than combination therapy. If antimalarial therapy with artesunate failed, patients were to be re-treated with combination artemisinin based anti-malarial therapy. Ethical permission for the study was given by the Committee for Human Research Publication and Ethics of the Kwame Nkrumah University of Science and Technology and the Komfo Anokye Teaching Hospital.

Study participants and study sites

Study participants were HIV-positive adult volunteers taking efavirenz-based cART (with >90% adherence) for a minimum of 2 weeks whose physician has decided to treat them for malaria with artesunate monotherapy based on complaints of symptoms of malaria at the chest clinic where HIV infected adults are routinely managed on out-patient basis. Controls who were patients not known to have HIV infection were recruited from the Polyclinic unit of the Komfo Anokye Teaching Hospital when their physicians had decided to treat uncomplicated malaria with artesunate monotherapy. Subjects were included if they were above 18 years, had given a written informed consent and willing to attend for study procedures. Patients were excluded if they had current or recent (within previous week) therapy with any known inhibitors or inducers of cytochrome P450 or P-glycoprotein, aside from antiretroviral drugs, pregnancy, current or recent therapy with traditional medicines (or intention to use such medicines) vomiting profusely or had known history malabsorption or if they required hospital admission. Both groups were treated with Artesunate 200 mg twice daily for 5 days. If patients showed signs or symptoms of failing to respond to antimalarial therapy at Day 5, the treating physician may decide to give an alternative antimalarial combination, in which case all subsequent data were not be used in the study, and the patient were given the option to withdraw from the study. Patients were recruited between May 2009 and January 2010.

Follow-up and sampling

After obtaining informed consent and after full explanation of the objectives of the study (in local language by a study nurse) a standard study Clinical Report Form (CRF) (see appendix 4) was used to record all data, including results of laboratory tests. The patients' medical and drug histories were taken prior to enrolment (Day 1). The first dose of Artesunate 200mg was administered under supervision. Drug adherence sheets were completed prior to the study and at each visit (Day 1 and Day 5), patients were requested to complete an adherence questionnaire and a symptom questionnaire. All subjects were fully examined and completed a symptom questionnaire at each visit. An initially proposed sampling schedule on day 1 at 0, 15, 30, 60, 90 and 120 minutes followed by further sampling at 4, 6, 8 and 12 hours were not acceptable to all the target HIV-infected and controls approached about the study. A revised pharmacokinetic sampling on day 1 at 0, 1, 4 and 6 hours after the first supervised dose of Artesunate 200mg and on day 5 at 6 hours post last dose was then agreed upon.

6ml of venous blood samples were collected at baseline and on Day 5 for malaria film, for full blood count and for biochemistry to assess the liver function test and renal function test. All plasma and serum samples were stored at -70^{o}C prior to transportation on dry ice to the UK. For determination of plasma efavirenz concentrations, blood samples were drawn at baseline (day 1) and on day 5 at the scheduled PK time points described for artesunate and DHA above. Plasma samples were transported to the Department of Pharmacology and Therapeutics (Liverpool, UK) for measurement of steady state plasma efavirenz concentrations and for Artesunate plus dihyroartemisinin at the Liverpool School of Tropical Medicine.

Blood smears for identification and enumeration of malaria parasites: Confirmation diagnosis of malaria and parasite count were made using thick and thin blood smears (day 1) prepared with peripheral blood, stained with Field's stain and Giemsa, respectively, and viewed in 100x microscopic fields. Parasite density was calculated by counting the number of asexual parasites against 200 leukocytes in the thick blood film by using a hand tally counter. If less than 10 parasites were found before reaching 200 leukocytes, then counting was continued until 500 leukocytes were counted. Parasite density (expressed as the number of asexual parasites per microliter) was calculated by dividing the number of asexual parasites by the number of leukocytes counted and then multiplying by an assumed leukocyte density of 8,000 leukocytes/µl.

Complete blood counts with haemoglobin concentrations, white blood cells and platelets counts were done centrally using Sysmex XT-1800i automated haematology analyser (USA). Serum biochemistry testing for liver and renal function tests were performed at the Chemical Department of KATH using the multi-parameter Roche Cobas Integra® 400 plus, Roche Diagnostics, Mannheim, Germany.

Drug Assays

Quantification of plasma artesunate and dihydroartemisinin concentrations in HIV infected patients and controls.

Plasma concentrations of Artesunate and its active metabolite Dihydroartemisinin (DHA) were determined by high performance liquid chromatography coupled to tandem mass spectrometry (HPLC-MS/MS) by a validated assay at the Liverpool School of Tropical Medicine and Hygiene. Plasma samples were heated at 58^{o}C for 1 hour to inactivate any residual HIV before measurements of Artesunate and Dihydroartemisinin concentrations could be performed.

Equipment and reagents

The Thermo Accela HPLC system consisted of Surveyor AS autosampler (200 vial capacity set at a temperature of 15^{0}C) and a Surveyor LC pump (Thermo Electron Corporation, Hemel Hempstead, UK). A Betasil (C_8) phenyl-hexyl column (5μm: 50mm x 2.1mm) connected/interfaced with a 10mm guard column of same packing material (Thermo Electron Corporation, Runcon, Cheshire, UK) set at an oven temperature of 26^{0}C was used to elute analytes and internal standard. The HPLC system was interfaced with a Thermo TSQ Quantum access triple-quadrupole mass-spectrometer with a positive electron spray ionisation scan mode. LC-MS grade acetonitrile (ACN) was obtained from Fisher Scientific (Loughborough, UK). Data acquisition and quantification were performed using Analyst 1.4 (Applied Biosystems/MDS SCIEX, Foster City, USA).

Analytical and Pharmacokinetic Methods

Plasma concentrations of Artesunate (ARTS) and Dihydroartemisinin (DHA) at various time points of the study were measured using a validated HPLC-MS/MS methodology. Patient samples were analysed in singlicate (100μl) as follows: Internal standard (IS) [deoxyartemisinin (50μl, 10μg/ml)] was added to all patient samples and serial dilutions of DHA standards followed by the addition of 200μl of precipitating solvent, ice cold acetonitrile. Samples were vortexed for 2-5 seconds and centrifuged at 13000rpm at 4^0C for 5 minutes. The solvent phase was then transferred into clean glass vials ready for injection (20μl) into the LC-MS system. The analytes were resolved on a betasil phenyl hexyl column using an isocratic mobile phase of 65:35 (vol/vol) of acetonitrile and 0.1M ammonium acetate at a flow rate of 0.45ml/min with a run time of 4 minutes and a wash out gradient. The Thermo TSQ Quantum access triple-quadrupole mass-spectrometer with a positive electron spray ionisation scan mode was used for the multiple reaction monitoring (MRM) LC-MS/MS analysis. The mass spectrometric conditions were optimized for the compounds by infusing a 100ng/ml standard solution in mobile phase at 10μl/min using a Harvard infusion pump connected directly to the mass spectrometer. An additional tuning optimization of gas flows and temperatures was performed by continuously infusing the same standard solution at 10μl/min via a "T" connector into the post-column mobile phase flow (500μl/min). The ionization source temperature was maintained at 475^oC and the ionization source voltage set at 4500V. The curtain gas was set to 25.0psi, and the nebuliser (GS1) and ionization source (GS2) gases at 55.0 and 60.0 psi, respectively. The CAD gas in the collision cell was set at 5 psi. DHA, ARTS and IS eluted at 1.27, 1.56 and 1.96 minutes respectively as shown in figure (Figure 2.1) and were detected by selected reaction monitoring (SRM). The intensities of the ammonium adducts of the parent compounds and fragment (daughter) ions mass-to-charge ratios [m/z], 302.2 and 267.0 for DHA, 402.2 and 267.0 for ARTS and 267.0 and 203.0 for deoxyartemisinin (shown in Figures 2A-C) were measured and total plasma concentrations were ascertained from the intensity of separate analyte: internal standard daughter ion ratios by comparison to the relevant calibrator of known concentration. The validated assay concentrations ranged from 5 – 2000ng/ml. Samples from both HIV-infected patients and controls were tested in one batch.

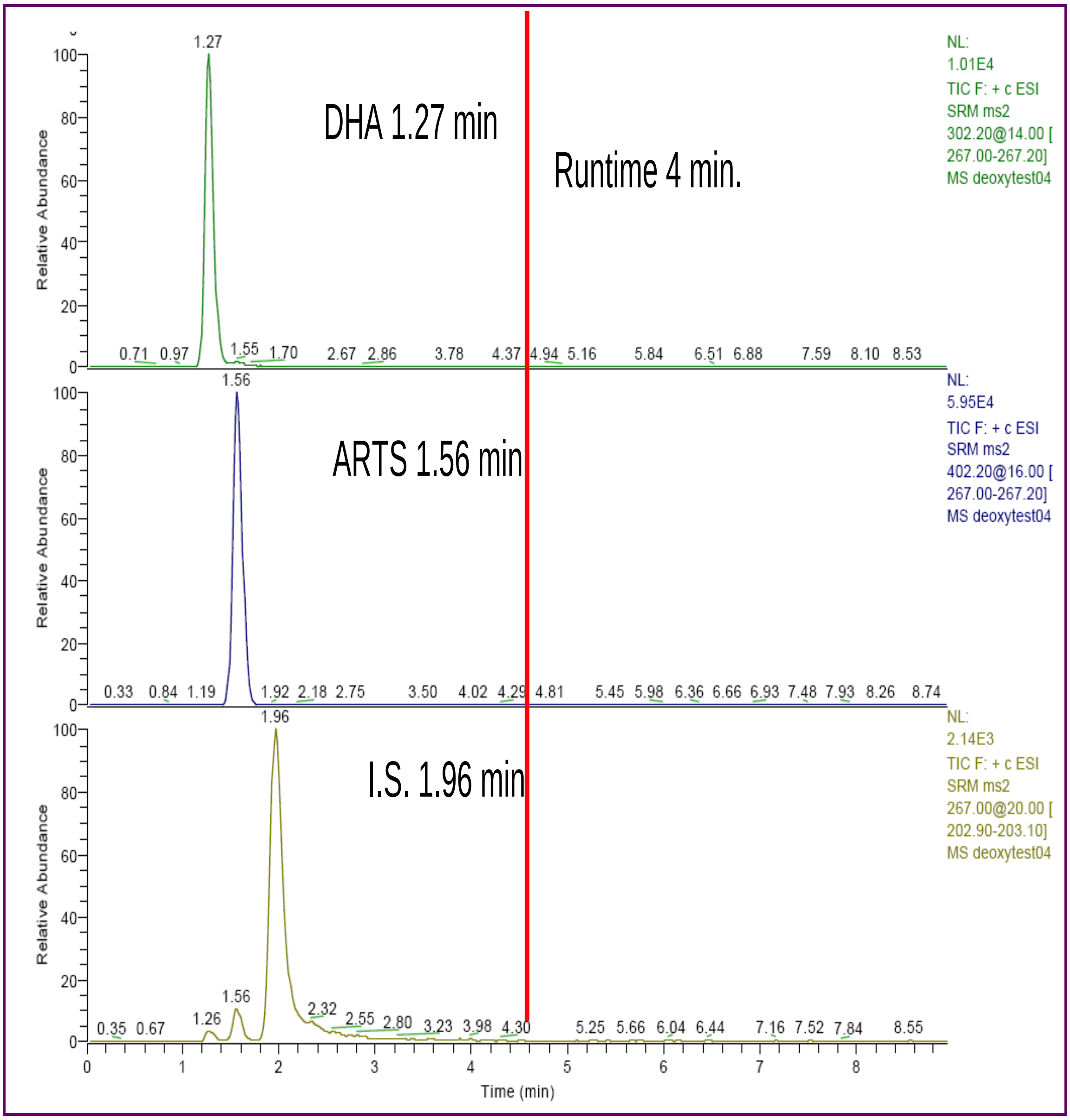


Figure 2.1. Chromatographic separation of Dihydroartemisinin (DHA), artesunate (ARTS) and internal standard (IS).

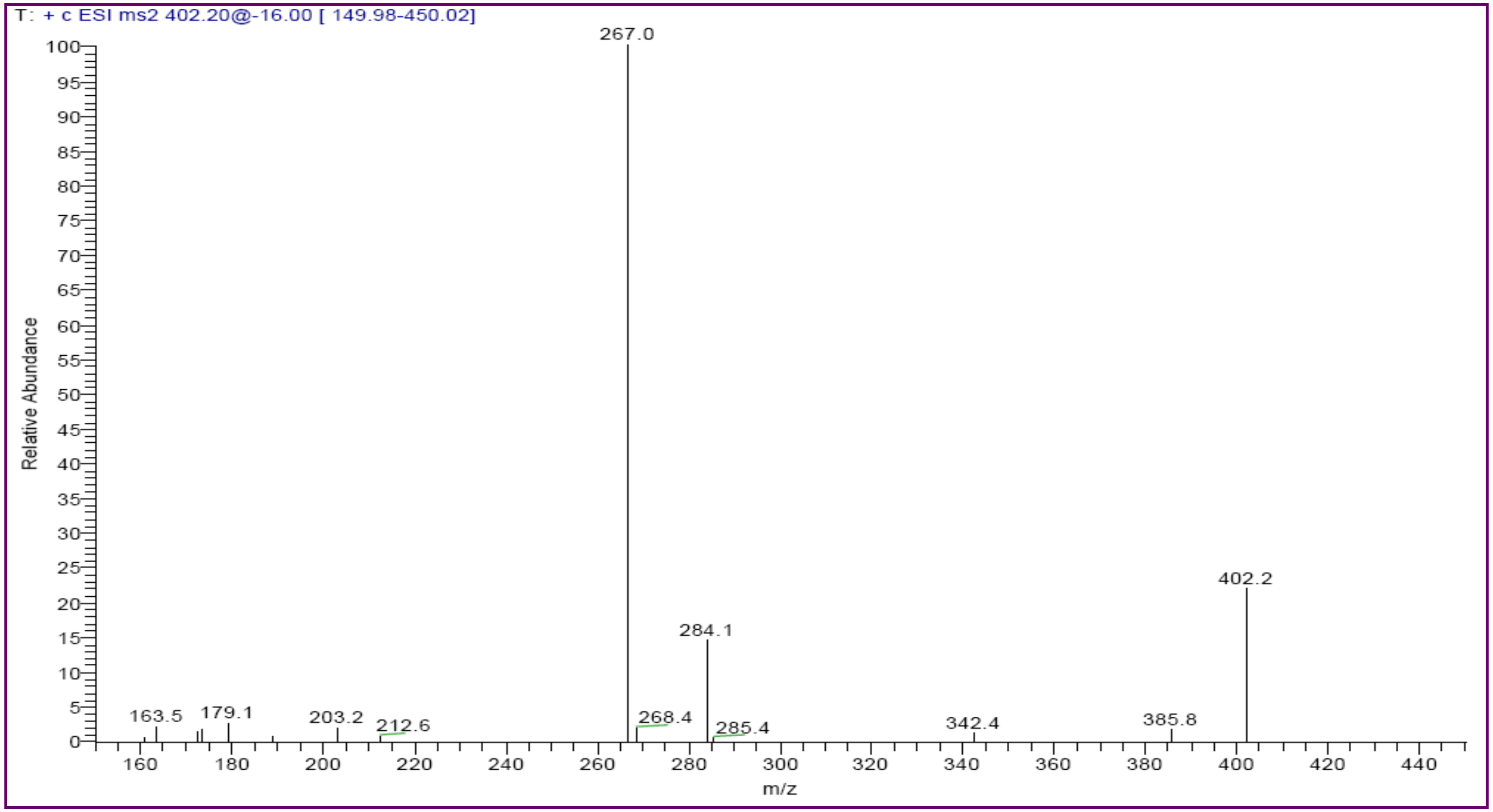


Figure 2.2A. Mass spectrometry of artesunate (ARTS) after direct injection in to mass-spectrometer, parent compound was observed at m/z 402.2 ($M+[NH_4]^+$) and major MS:MS fragmented ion of parent molecule was observed at m/z 267.0

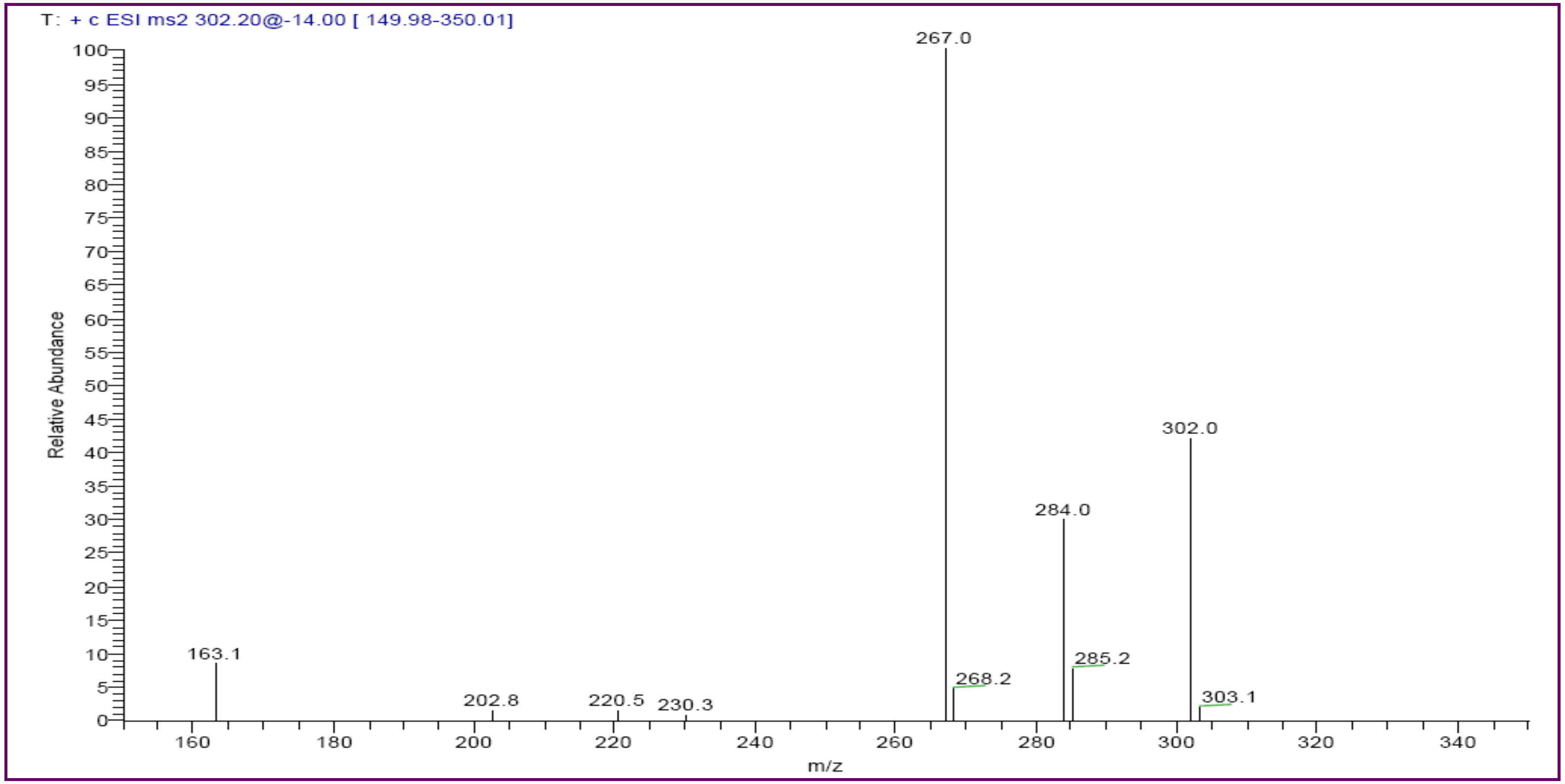


Figure 2.2B. Mass spectrometry of dihydroartemisinin (DHA) after direct injection in to mass-spectrometer, parent compound was observed at m/z 302.2 ($M+[NH_4]^+$) and major MS:MS fragmented ion of parent molecule was observed at m/z 267.0.

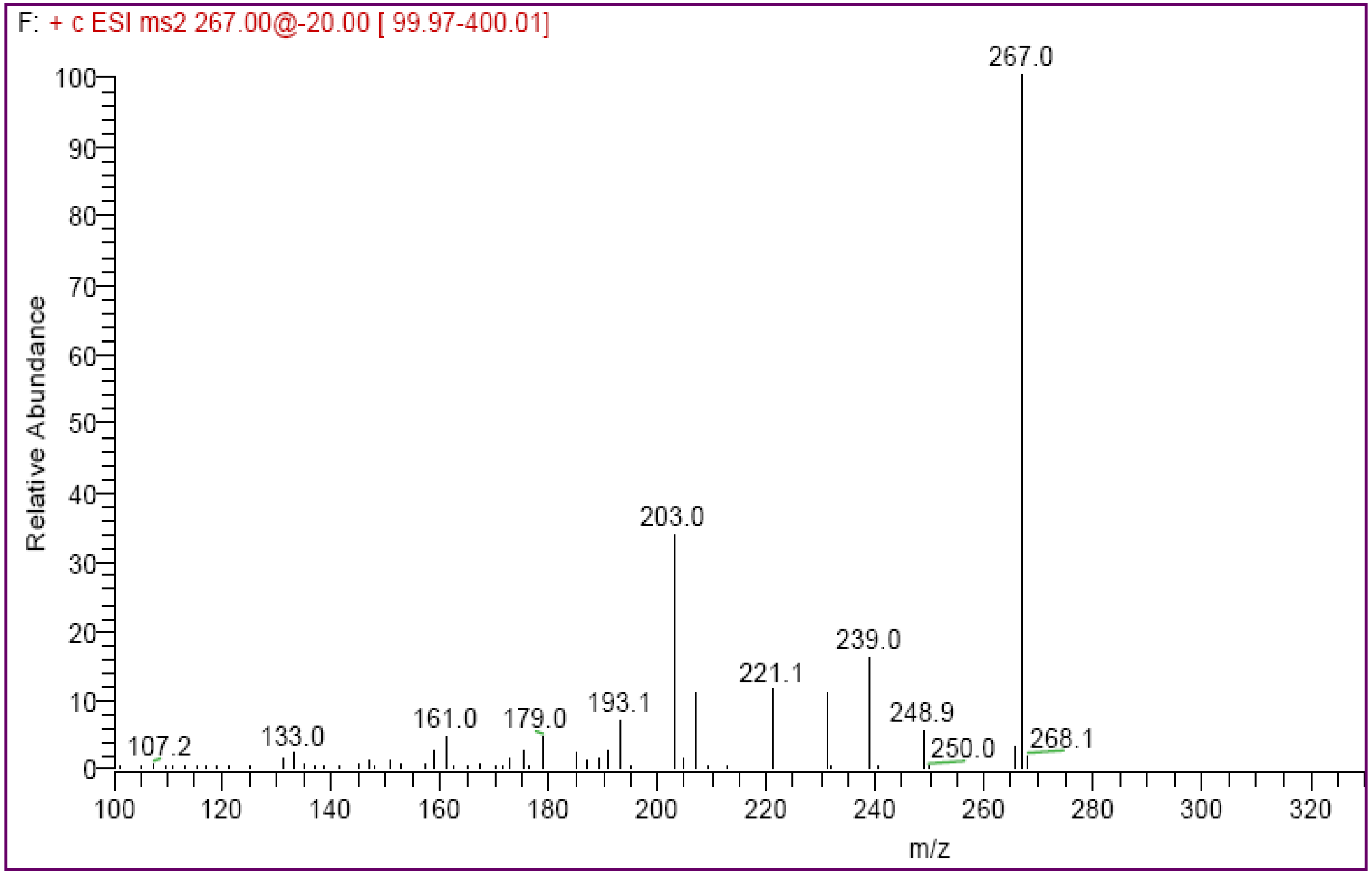


Figure 2.2C. Mass spectrometry of deoxyartemisinin (IS) after direct injection in to mass-spectrometer, parent compound was observed at m/z 267.0 ($M+H^+$) and major MS:MS fragmented ion of parent molecule was observed at m/z 203.0. IS= internal standard

Quantification of plasma concentrations of efavirenz

Plasma efavirenz concentrations were measured as using HPLC coupled with UV detection as described under methods under section 2.8.

Pharmacokinetic and statistical analysis

The pharmacokinetic parameters tested were area under the curve, time to maximum concentration (T_{max}) and maximum concentration (C_{max}) for dihyroartemisinin (DHA). The area-under-curve from time zero to 6 hours (AUC_{0-6}) was calculated using the linear trapezoid rule without extrapolation beyond this time period for dihydroartemisinin. The effect of artesunate on efavirenz steady state concentration was assessed by determining the percentage change in the concentration at times 1, 4, 6 hours after the first dose of Artesunate and also 6 hours after the last dose of Artesunate on day 5 compared with time 0 hours on the first day. Medians and means between cases and controls were compared using either Mann-Whitney's U-test or student's t-test respectively. Pharmacokinetic modeling of Dihydroartemisinin concentrations in serum over the first 6 hours was performed using the GraphPad 4 Prism software. No adjustments were made for multiple comparisons.

2.8.1. Methods for chapter eight

2.8.1.1 *Study participants*

800 patients with confirmed HIV infection were recruited into the first part of the study evaluating the frequencies of common SNPs along the metabolic axis of efavirenz in our study population. In the second part, subset of patients on efavirenz based cART with genotype data were analysed to examine the impact of selected polymorphisms in efavirenz metabolising enzymes on steady state concentrations of plasma efavirenz. To be included in the study, patients should be aged at least 15 years and those on efavirenz should be taking it at the fixed dose 600mg daily plus two nucleoside reverse transcriptase inhibitors based on pharmacy records.

2.8.1.2. Pharmacokinetic sampling: Mid-dose sampling is frequently used in clinical studies of efavirenz disposition for patient convenience as the drug is invariably taken at bedtime to minimize central nervous system side effects during the day[8]. Mid-dose blood samples were obtained from patients at the Serology department of KATH where patients routinely go for phlebotomy to obtain samples for CD4 count enumeration. Plasma and sera were separated by centrifugation at 4^0C, aliquots were collected and stored at -20^0C. Samples for this study were selected from plasma and sera repository in Kumasi set up for HIV research since 2005. Patients who were on efavirenz-based cART from pharmacy records had their samples traced from this repository for the present analysis. Samples were shipped from Ghana to the Department of Pharmacology and Therapeutics of Liverpool University, United Kingdom on dry ice.

Upon receipt, plasma samples were heat inactivated in a water bath at 58^0C for 40 minutes and stored at -20^0C prior to further analysis.

2.8.1.3. Quantification of plasma efavirenz concentration

Plasma concentrations of efavirenz was measured using reverse phase high performance liquid chromatography (HPLC) with UV detection of efavirenz at 250nm by a validated assay at the Liverpool University, department of Pharmacology and Therapeutics. The laboratory at the University of Liverpool participates in an external international quality assurance scheme (KKGT, The Netherlands). The methodology for the preparation of plasma samples for quantification of efavirenz is described below:

Equipment: Efavirenz concentrations were determined by HPLC-UV. The HPLC system consisted of a HPLC 465 autosampler and a 325 pump (Kontron Instruments Ltd; Hertfordshire, UK) and a Spectra System UV1000 detector (Thermo Separation Products; Hemel Hempstead) set at wavelength of 250nm. A Hypurity C_{18} column (5µm 150 x 4.6mm, Thermo Electron Corporation) was used to elute analytes and internal standard, and was interfaced with a 2µm guard column (Si 60, 5µm; Merck, Germany). Peak integration, data acquisition and processing were performed using Chromeleon Software (Version 6.40, Dionex UK Ltd, Surrey, UK).

Reagents: HPLC grade de-ionised water was produced from an Elga Option 4 water purifier (Elga LabWater, High Wycombe, UK). Analytical grade acetonitrile (ACN), ethyl acetate, n-hexane and methanol were obtained from VWR International Ltd. Drug free plasma was obtained from the National Blood Service (Liverpool, UK).

Analytical Methods: 200μl of singlicate patient samples, quality control samples and blank plasma containing serial dilutions of efavirenz standards were carefully pipetted into glass tubes and spiked with 20μl of 250μg/ml of an internal standard [Ro 31-9564, Roche Discovery Welwyn, UK]. To this was added 100μl of K_2CO_3 (approximate pH of 11) to enhance the efficiency of EFV extraction following which 3ml of an extraction solvent comprising of 50:50 (v/v) ethyl acetate: n-hexane was also added. All samples were thoroughly mixed on a rotary mixer (Rotadrive STR4, Stuart Scientific) for 30 minutes and then centrifuged for 5 minutes at 4000 rpm. The lower aqueous phase was frozen in a cryogenic bath (of dry ice in methanol) and the upper organic phase transferred into fresh 5ml glass tubes and evaporated to dryness in a rotary evaporator (RC10.22, Jouan). Samples were reconstituted in 150μl of mobile phase (50:50 [v/v]; deionised water with 10mM ammonium acetate buffer: acetonitrile), vortexed and then transferred to autosampler vials ready for injection (50μl) onto the HPLC column. Efavirenz and internal standard were resolved on Hypurity C_{18} column (5μm 150 x 4.6mm, Thermo Electron Corporation) with an isocratic mobile phase (50:50 [v/v]; deionised water: acetonitrile at a flow rate of 1.2ml/min. EFV was eluted over 20 minutes and its concentration derived by dividing its integrated area under the curve by that of the internal standard. The lower limit of quantification was 99ng/ml (± 2SD), and inter and intra-assay variability was <10% and <10% respectively.

2.8.1.4. Genotyping of CYP2B6, CYP2A6, UGT2B7 and CAR SNPs

DNA extraction from serum samples: Before extracting genomic DNA from sera of patients, samples were brought to room temperature (15-25^{0}C) and heated in a water bath at a temperature of 56^{0}C to inactivate HIV that may be present in these samples.

DNA was extracted from sera samples using a Qiagen DNA extraction mini kit according to manufacturer's instructions. Briefly, 60μl of Qiagen protease was pipetted into a 1.5ml sterile eppendorf tube followed by the addition of 600μl of a well-mixed serum sample and 600μl of lysis buffer AL. The resultant 1260μl solution was pulse - vortexed for 15 seconds, incubated at 56^0C for 10 minutes, spun briefly for 1 minute at 6000 x g and divided into 2 sterile eppendorf tubes. Into the contents of each of these two tubes 300μl of 100% ethanol was added and pulse-vortexed for 15s, spun at 6000 x g for 1 minute, carefully transferred to a spin column without wetting the rim of the filter and spun again at 6000 x g for 1 minute. After discarding the filtrate, the DNA on the filter membrane was purified by placing the filter unto a new clean collection tube, adding 500μl of wash buffer AW1 to the spin column and spinning at 6000 x g for 1 minute. The purification step was repeated using 500μl of wash buffer AW2 followed by centrifugation at 13,000g for 3 minutes to dry the membrane completely. Cleaned DNA from the filter membrane was eluted into a new sterile eppendorf tube by carefully applying 50μl of elution buffer AE to the filter membrane, incubating at room temperature for 3 minutes and spinning at 6000 x g for 1 minute. Elution was performed twice and genomic DNA stored at -20^0C for later analysis.

a. CYP2B6 genotyping: Pre-amplification for exon 4 and exons 7 and 8 (combined) was first conducted to discriminate from the CYP2B6 pseudogene (CYP2B7). Genotyping for G516T and T983C was then performed on the resultant amplicons by real-time PCR allelic discrimination. Briefly 1μl of genomic DNA was added to a PCR mix comprising 1μl of 10x buffer II (Applied Biosystems), 0.6 μl of 25mM $MgCl_2$ (Applied Biosystems), 0.4 μl each of Exon4 forward (1μM) and reverse (μM) primers, 0.2μl of

10μM dNTP (dATP, dCTP, dGTP, dTTP) (Applied Biosystems), 0.2μl of amplitaq 5u/μl (Applied Biosystems) and 6.2μl of sterile water. E4 forward and reverse primer sequences were 5′-GGTCTGCCCATCTATAAAC-3′ and 5′-CTGATTCTTCACATGTCTGCG-3′ respectively. PCR conditions were 95^0C for 5 minutes followed by 45 cycles of 30s at 95^0C, 30s at 58^0C, 45s at 72^0C and a final stage of 72^0C for 5 minutes before holding at 4^0C in a thermocycler (Applied Biosystems). Pre-amplification of exons 7 and 8 combined was performed under similar conditions: Exon 7 forward primer sequence was GTGATTATTCATTAATTGGGTTC and Exon 8 reverse primer sequence was TGCAATGGTTGATTGATGCTC.

Genotyping for 516G>T and 983T>T was then performed on the resultant amplicons by real-time PCR allelic discrimination. Briefly 2μl of amplicon material was added to a PCR mix of 12.5μl of ABgene QPCR master mix (2X) (Abgene, Epsom, UK), 1.25μl of primer mix (20X) (Abgene, Epsom, UK), 1.25μl of probe mix (20X) (Abgene, Epsom, UK) and 8μl of sterile water (Sigma, UK) in a white MJ plate (Biorad laboratories Ltd, UK) and sealed with an Abgene plate seal. Plate was spun briefly at 1300g following which PCR was performed on a qPCR-Opticon® monitor (MJ Research Inc., USA). PCR conditions were 95^0C for 5 minutes followed by 50 cycles of 95^0C for 15s and 60^0C for 1 minute and a plate read with the FAM and VIC dyes reporting T and G respectively for 516G>T while the FAM and VIC dyes reported T and C respectively for 983T>C. G516T-GTOTF forward primer sequence was CTTGACCTGCTGCTTCTTTCCTA and the G516T-GTOTR reverse primer sequence was AGACGATGGAGCAGATGATGTTG. G516T-GTOT VIC sequence was TTCCAGTCCATTACCG and G516T-GTOTM1 FAM sequence was

TTCCATTCCATTACCG. CYP2B6E7-TTOCF forward primer sequence was GCCTGAAATGCCTCTTTAAAATGAGATTC and the CYP2B6E7-TTOCR reverse primer sequence was GCGATGTGGGCCAATCAC. T983C-TTOCV2 VIC sequence was CTGTTCAGTCTCCC and the T983C-TTOCM2 FAM sequence was CTGTTCAATCTCCC.

b. CYP2A6 genotyping: The CYP2A6 allele genotyped was the CYP2A6*9B by real-time PCR allelic discrimination. A similar procedure was employed as for that described for the CYP2B6 with the following exceptions:

genomic DNA material was used for the real-time PCR without a pre-amplification step.

- 40 cycles of qPCR was performed instead of 50 for the 2 CYP2B6 alleles described above
- FAM and VIC dyes reported C and A for the CYP2A6*9B-A/C (rs8192726) SNP respectively.

*c. UGT2B7 genotyping***:** Genotypes for the UGT2B7 exon 2 SNPs namely UGT2B7*1A and UGT2B7*2 were determined by real-time PCR allelic discrimination. The protocol was similar to that described for CYP2A6 above with the following exceptions:

- FAM and VIC dyes reported T and C for the UGT2B7*2_802C>T SNP and G and A for the UGT2B7*1A_735A>G SNP respectively.
- UGT2B7_802_F forward primer sequence was CTGACGTATGGCTTATTCGAAACTC and the UGT2B7_802_R reverse primer sequence was TGGAGTCCTCCAACAAAATCAACAT. UGT2B7_802 VIC sequence

was AGTGGATGAGGAAACTT and UGT2B7_802 FAM sequence was AGAGTGGATAAGGAAACTT.

- UGT2B7*1A_735_F forward primer sequence was CCTAAAGTAATTATCTTGTGTCATCCACCTT and the UGT2B7*1A_735_R reverse primer sequence was CGTCAGCTTTCCCCATTGTCT. UGT2B7*1A_735 VIC sequence was ACCCACTACATTATCTGT and the UGT2B7*1A_735 FAM sequence was CCCACTACGTTATCTGT.

d. Constitutive Androstane Receptor (CAR) genotyping: Genotyping for the C>T (rs2307424) SNP for CAR was conducted by real-time PCR using a pre-validated Applied Biosystems assay ID C_25746794_20.

Analysis of real-time PCR data: After PCR amplification, the endpoint plate read was performed using an Applied Biosystems Sequence Detection System (SDS). The SDS software uses the fluorescence measurements made during the plate read to plot fluorescence (Rn) values based on the signals from each well. The plotted fluorescence signals indicated which alleles are in each sample. A cycling threshold for each dye was set as shown in figure 2.3. An allelic discrimination plate read document is set up on the SDS instrument to analyse the plate read document to either make manual allele calls or review automatic allele calls followed by conversion of allele calls to genotypes as shown in figure 2.4.

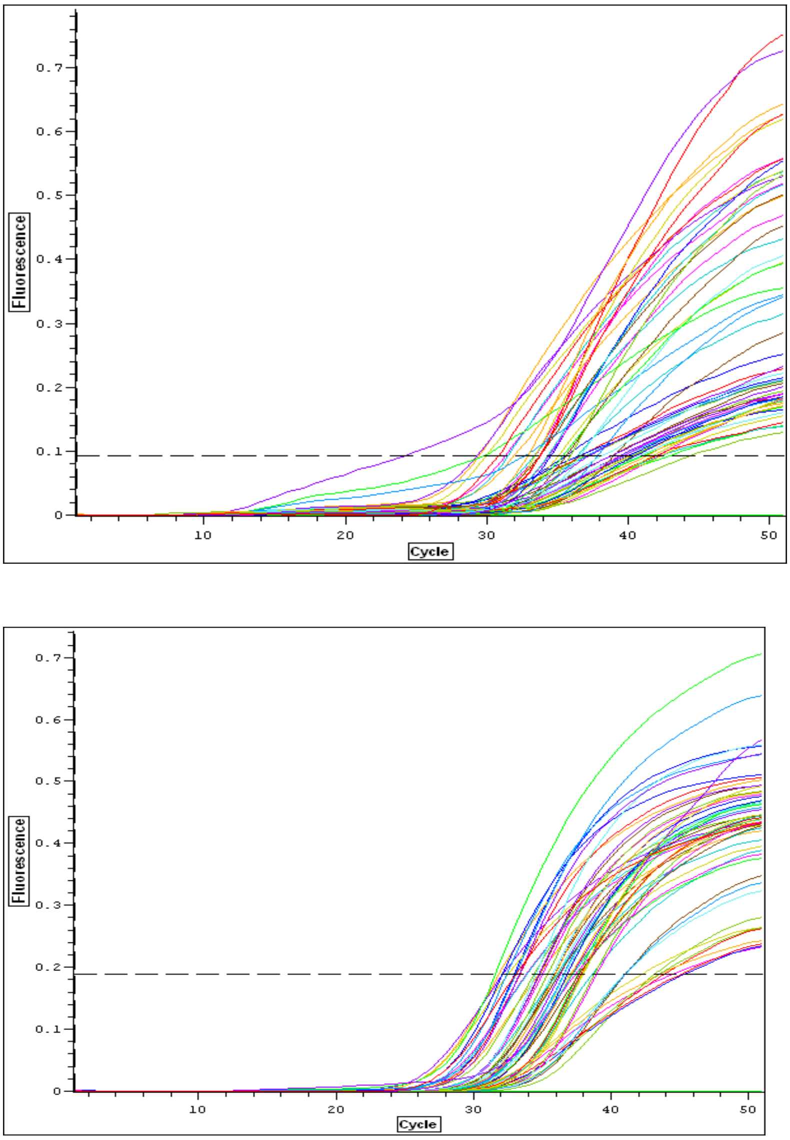


Figure 2.3. Real-time PCR results in FAM (upper pane) and VIC (lower pane) dyes after 50 cycles for UGT2B7_802. Cycling threshold (broken lines) set manually at fluorescence intensity of 0.1 and 0.2 for FAM and VIC dyes respectively.

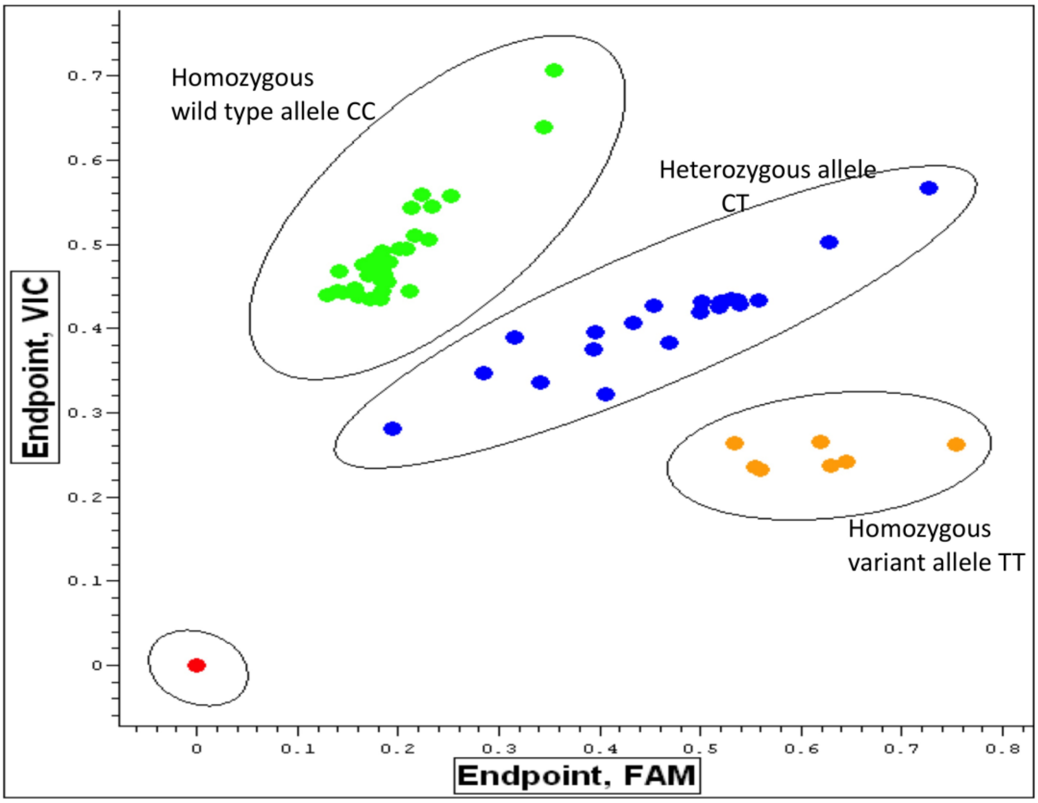


Figure 2.4 showing the genotypic read out of one run of real time PCR of 57 samples for UGT2B7*2. The VIC dye signalled the wild type allele C while the FAM dye signalled the mutant allele T of the UGT2B7 SNP at position 802. There were 30 patients with normal genotype CC, 20 with heterozygous genotype CT and 7 with homozygous variant allele TT.

2.8.1.5 Statistical analysis (chapter 8)

The concentration of serum efavirenz was log transformed to achieve normalisation and equal variance. Chi-squared test was employed to assess whether the observed and expected genotype frequencies were in Hardy –Weinberg equilibrium. Univariate statistical analyses examining the effects of patient demographics and genotypes on the efavirenz mid-dose concentrations were assessed by Mann-Whitney U-test (for gender and for two genotype groups), analysis of variance (ANOVA) (for three genotype groups) or by simple linear regression (continuous data such as age, body weight, height and body mass index (BMI) using Pearson's r correlation coefficient. A two-way ANOVA, which incorporates an interaction model was employed to evaluate the effects of gene-gene interactions among the selected SNPs. Where the ANOVA (one-way or two-way) yielded a significant effect ($p<0.05$), a post hoc multiple pair-wise comparison testing was performed using the Mann-Whitney's U-test to identify groups that were significantly different from each other. A multivariate analysis was then conducted to construct a predictive model using patient demographic, anthropometric and genotypes as independent variables and efavirenz concentrations as the dependent variable. Dichotomous variables, including gender, CYP2B6 983T>C, CYP2A6*9B, CAR C>Trs2307424 genotypes were coded as 0 or 1 while CYP2B6 516G>T; UGT2B7_735A>G and UGT2B7_802C>T were coded as 0,1 and 2 for homozygous wild type, heterozygous mutants and homozygous mutants respectively while age, weight, height and BMI were included as continuous variables. Thus genotypes with rare double mutants (defined by < 3 in the total sample population) were treated as one genotype with the heterozygous mutant in these analyses. A multiple linear regression using best subset selection was then performed with stepwise removal of non-significant variables until all remaining independent variables had coefficients with p-values<0.05. The risks of immunological failure and central nervous system toxicity on efavirenz-based cART in relation to selected SNPs and mid-dose efavirenz were assessed using a Kaplan-Meier analysis. Statistical analyses were performed using the SPSS version 17 and Prisms Graphpad4.

CHAPTER THREE

A descriptive analysis of baseline characteristics, vital status, risk factors for renal impairment and elevated transaminases among HIV-infected Ghanaians initiating cART in Kumasi, Ghana.

Introduction

Human immunodeficiency virus (HIV) infection is still a global public health issue, contributing to approximately more than 2 million deaths each year. Sub Saharan Africa remains by far the worst affected region, harbouring more than two thirds of HIV-infections worldwide. Scaling up access to treatment is one priority of the worldwide activities in the fight against the HIV epidemic. An increase of 1.2 million people, or 30%, was achieved only in 2009. Still, of about 15 million people in need of ART in low- and middle-income countries, only about 36% (5.2 million) were receiving ART at the end of 2009[1]. At the same time, the numbers of patients who have been on ART for 5 years and more is increasing, and thus the number of patients at risk for failure of first-line ART, is increasing rapidly.

It was clearly demonstrated that ART in resource poor settings can be successful. Therapy outcomes in Sub Saharan treatment programmes were mainly reported to be favourable and comparable to the levels achieved in industrialised countries[1, 364-368]. Large scale ART roll-out in Sub Saharan Africa was launched only 2002/2003, and absolute numbers of patients newly started on ARV are still increasing. Thus, the drawbacks of the ART rollout are also expected to become evident only with delay compared to industrialised countries, where HAART was introduced in 1996. However, data on the long term efficacy of ART in Sub Saharan Africa is scarce and initial optimistic reports on the success rates of ART programmes are debatable considering short follow-up periods, high rates of loss to follow-up (LTFU) and deaths.

In Ghana, around 3% of the population is HIV infected and since January 2004, highly-subsidised antiretroviral therapy (ART) has become available through the Global Fund for Infectious Diseases and Family Health International, via the National AIDS Control Programme in Ghana. Whilst only around 3% of the population in Ghana is infected with HIV-1, a relatively large proportion (5-10%) is infected, either alone or in addition to HIV-1, with HIV-2. Kumasi is the second largest city in Ghana with a population of 2 million. The Komfo Anokye Teaching Hospital (KATH) is the main university-affiliated teaching hospital in Kumasi, which provides HIV care through the Department of Medicine, and over 11,000 patients have now been enrolled at KATH as at end of 2011, of which around 5,000 have started a predominantly NNRTI-based ART.

This chapter introduces the patients who initiated cART as prelude to chapters 4 to 6 within which the long-term effectiveness of NNRTI-based cART among Ghanaian HIV-infected patients have been evaluated. The aim of this chapter is therefore to describe the baseline characteristics of 4,039 HIV infected Ghanaians initiating anti-retroviral therapy in a busy out-patient clinic in a tertiary referral hospital between January 2004 and 31st December 2010. The focus is to highlight the demographic characteristics of HIV infected patients in this cohort, describe the burden of HIV associated disease prior to initiation of therapy, describe baseline risk factors associated with HIV-related kidney disease and HIV-associated hepatic injury. Baseline demographic, clinical and laboratory parameters are compared in patients initiating an efavirenz, nevirapine or a protease inhibitor based anti-retroviral therapy. Data on sero-prevalence of Hepatitis B co-infection are also provided and its impact on ART outcomes and risk for hepatoxicity on ART are analysed in subsequent chapters of this dissertation. To gain further insight into the vital status of patients lost-to-follow up, a telephone survey was conducted among a proportion of patients or their contacts. Finally, a preliminary analysis of baseline factors of patients initiating ART influencing the vital status at the time of censoring data for analysis are provided as an introduction for the subsequent chapters of this thesis.

Methods

Please refer to chapter 2 sections 2.3.

Results

Demographic characteristics of patients initiating ART in Kumasi, Ghana.

Four thousand and thirty-nine (4,039) adult patients were initiated on anti-retroviral therapy between January 2004 and December 2010 in Kumasi, Ghana. There were 1,287 male and 2,752 female patients with a preponderance of females with a female to male ratio of 2.1:1.0. Table 3.1 shows the age and gender distribution of study participants with an overall median (range) age of 38 (14 – 77) years. Male patients were significantly older than female patients initiating ART with a median (range) age of 41 (14 – 77) years versus 37 (14 – 76) years respectively, $p<0.0001$ by Mann-Whitney, U-test.

Table 3.1. Age and gender distribution of HIV infected Ghanaians initiating ART in Kumasi, Ghana.

Age range (years)	Male n (%)	Female n (%)	Total n(%)
≤ 20	10 (0.8)	35 (1.3)	45 (1.1)
21 - 30	94 (7.3)	617 (22.4)	711 (17.6)
31 – 40	515 (40.0)	1,170 (42.5)	1,685 (41.7)
41 – 50	484 (37.6)	628 (22.8)	1,112 (27.5)
51 – 60	146 (11.3)	236 (8.6)	382 (9.5)
>60	38 (3.0)	66 (2.4)	104 (2.6)
Sub-total	1,287 (100.0)	2,752 (100.0)	4,039 (100.0)

Table 3.2 and Figure 3.1 shows the regional / geographical distribution of patients accessing care at the clinic situated in Kumasi, the regional capital of the Ashanti region of Ghana. A significant proportion of patients were from the Ashanti region (93%), followed by the Brong-Ahafo, Central and Western regions respectively. Of the 3,768 cases from the Ashanti region initiating therapy, 75% of cases were from the Kumasi metropolitan city with the remainder coming from major district capitals such as Obuasi (3%), Offinso (1%), Ejisu (1%) and so forth.

Table 3.2. Regional distribution of patients accessing ART in Kumasi, Ghana.

Region	Number of patients	% of total
Ashanti	3,768	93.3
Brong-Ahafo	88	2.2
Western	33	0.8
Central	23	0.6
Upper East	19	0.5
Northern	9	0.2
Eastern	5	0.1
Upper West	4	0.1
Greater Accra	1	0.0
Volta	1	0.0
No data	88	2.2

Figure 3.1. The map of Ghana showing the 10 administrative regions with their regional capital cities/towns. Kumasi is the capital city of the Ashanti region where the HIV clinic is situated.

HIV associated morbidity before initiation of therapy.

Two hundred and seventy-three (273) patients corresponding to 6.8% of the total number of patients were in clinical stage 1, 487(12.1%) were in clinical stage 2 while 2,164 (53.6%) and 646 (16.0%) patients were in clinical stages 3 and 4 respectively prior to initiation of ART as shown in Table 3.3. Proportionally, there were no significant differences in gender with respect to severity of disease. Nearly 12% of patients had no data on their clinical stages prior to initiation of therapy.

Table 3.3. WHO clinical staging of patients according to gender.

WHO Clinical stage	Male n (%)	Female n (%)	Total n (%)	Chi-squared test, p-value
1	75 (5.8)	198 (7.2)	273 (6.8)	3.68, p=0.45
2	153 (11.9)	334 (12.1)	487 (12.1)	
3	687 (53.4)	1,477 (53.7)	2,164 (53.6)	
4	214 (16.6)	432 (15.7)	646 (16.0)	
No data	158 (12.3)	311 (11.3)	469 (11.6)	
Total	1,287 (100.0)	2,752 (100.0)	4,039 (100.0)	

Table 3.4 summarises the morbidity of HIV associated opportunistic infections and malignancies before initiation of therapy. Only 678 patients corresponding to a frequency of 16.8 per 100 persons were asymptomatic before starting treatment. The commonest manifestation of HIV disease was significant weight loss, present at a frequency of 30.8 per 100 persons, followed by oro-oesophageal candidiasis at a combined frequency of 15.5 per 100 persons and then tuberculosis (including current, previous, pulmonary and extra-pulmonary) at a frequency of 11.5 per 100 persons. Muco-cutaneous manifestations of HIV infection occurred at a frequency of 12.7 per 100 persons with a non-specific non-pruritic, hyperpigmented skin rash being the commonest cutaneous disorder followed by herpes zoster and pruritic papular dermatitis. Nervous system disorders were clinically detected and recorded at a frequency of 4.0 per 100 persons and included entities such as cerebral toxoplasmosis manifesting predominantly as either seizure disorder or motor weakness, AIDS dementia complex and CMV retinitis.

The body mass index (BMI) of patients at initiation of cART followed a normal distribution. The mean ± SEM BMI of patients prior to initiation of cART was 20.3 ± 0.07 with 35% of patients with BMI below the ideal body mass index of 18.5 kg/m^2. The mean ± SEM BMI in males of 20.3 ± 0.08 kg/m^2 was comparable to that in females of 20.2 ± 0.10 kg/m^2, p=0.7 by student's unpaired t-test.

Table 3.4. Frequency of HIV associated opportunistic infections and malignancies among 4,039 Ghanaian HIV infected patients initiating cART.

Conditions	Number of patients	Frequency /100 patients
Asymptomatic	678	**16.8**
Constitutional disorders		
Weight loss>10%	1,245	30.8
Wasting syndrome	123	3.0
Prolonged fever	10	0.3
Progressive generalised lymphadenopathy	10	0.3
Others	12	0.3
Sub-total	**1,400**	**34.7**
Gastrointestinal disorders		
Chronic diarrhoea	422	10.5
Oesophageal candidiasis	321	7.9
Oral candidiasis	307	7.6
Ano-rectal lesions	12	0.3
Others	24	0.6
Sub-total	**1,086**	**26.9**
Pulmonary disorders		
Pulmonary tuberculosis on therapy	284	7.0
Chronic cough (cause unknown)	203	5.0
Pulmonary tuberculosis previously	145	3.6
Bacterial pneumonia previously	80	2.0
Extrapulmonary tuberculosis	36	0.9
Others	7	0.2
Subtotal	**755**	**18.7**
Muco-cutaneous disorders		
Non-specific dermatosis	169	4.2
Herpes zoster	150	3.7
Pruritic popular eruptions	97	2.4
Kaposi sarcoma	57	1.4
Others	41	1.0
Subtotal	**514**	**12.7**
Nervous system disorders		
Cerebral toxoplasmosis	55	1.4
AIDS Dementia Complex	29	0.7
CMV retinitis	25	0.6
Peripheral neuropathy	15	0.4
Others	36	0.9
Subtotal	**160**	**4.0**

Medical co-morbidities present before initiation of cART included systemic arterial hypertension at a frequency of 2.0 per 100 persons, diabetes mellitus at 0.9 per 100 persons, bronchial asthma, sickle cell disease and cardiac disorders such as rheumatic heart disease, ventricular septal defect and one patient on cardiac pacemaker on account of HIV associated cardiomyopathy as shown in Table 3.5.

Table 3.5. Frequency of medical co-morbidities among 4,039 Ghanaian HIV-infected patients initiating cART.

Condition	Number of cases	Frequency/100 patients
Systemic arterial hypertension	79	2.0
Diabetes mellitus	36	0.9
Bronchial asthma	11	0.3
Sickle Cell Anemia	5	0.1

Laboratory characteristics of patients at initiation of cART

Baseline CD4 counts of patients did not follow a normal distribution curve. The overall median (range) CD4 T-cell count before ART was 134 (0 – 1,134) cells/mm^3 with median CD4 T-cell count in female patients significantly higher than that of males, 147 (0 – 1,134) vs 108 (1 – 789) respectively, $p<0.0001$. As expected the median CD4 T-cell count was significantly higher for patients with early clinical stages of HIV disease compared to those with later advanced disease: those with stage 1,2,3 and 4 disease had median (IQR) CD4 counts of 219.0 (143.0 – 296.0), 160.0 (80.0 – 226.0), 124.0 (47.0 – 205.0) and 82.0 (20.0 – 180.0) cells/mm^3 respectively with $p<0.0001$ by Kruskal Wallis test.

The median (range) haemoglobin concentration before ART overall was 10.2 (2.6 – 19.8 g/dl, n=3918). 75% of patients had various degrees of anaemia before ART – 34% had mild anaemia, 37% had moderate anaemia and 14% had severe anaemia - while only 15% had no anaemia. The median (IQR) haemoglobin concentration in males of 10.6 (9.0 – 12.2 g/dl) was significantly higher than that of female patients of 10.3 (9.3 – 11.4 g/dl), p=0.02.

HIV-related kidney disease: Baseline serum creatinine measurements were available for 3,137 (77.7%) of patients of which eGFR could be calculated using the Cockroft Gault, MDRD and CKD-EPI formulae for 3,054 (97.4%), 3130 (99.8%) and 3130 (99.8%) patients respectively as shown in table 3.6. There was a very good agreement between stages of renal impairment assessed using eGFR calculated using MDRD and CKD-EPI with a κ-value (95% CI) of 0.94 (0.93 to 0.95) and a weighted κ-value of 0.96. The agreement between stages of renal impairment estimated using the Cockcroft-Gault and either the MDRD or CKD-EPI showed fair degrees of agreements with κ-values 0.23 and 0.22 respectively and moderate agreements in linearly weighted kappa(Table3.6).

Table 3.6. Frequency of stages of renal impairment estimated by creatinine clearance using Cockroft-Gault, MDRD and CKD-EPI equations.

Stage of renal impairment		Frequency (%) by Cockroft-Gault	Frequency (%) by MDRD	Frequency (%) by CKD-EPI	κ statistic, (95% CI), Weighted κ CG vs MDRD	κ statistic, (95% CI), Weighted κ CG vs CKD-EPI	κ statistic (95% CI); Weighted κ CKD-EPI vs MDRD
Stage 1	>90 ml/min	693 (22.7)	1636 (52.3)	1696 (54.2)	0.23	0.22	0.94
Stage 2	60-89ml/min	1175 (38.5)	1065 (34.0)	1000 (31.9)	(0.21-0.26);	(0.20-0.25);	(0.93 - 0.95);
Stage 3A	45-59ml/min	644 (21.1)	241 (7.7)	231 (7.4)	0.418	0.410	0.959
Stage 3B	30-44ml/min	368 (12.0)	92 (2.9)	106 (3.4)			
Stage 4	15-29ml/min	130 (4.3)	62 (2.0)	61 (1.9)			
Stage 5	<15 ml/min	44 (1.4)	34 (1.1)	36 (1.2)			
Total		3,054	3,130	3,130	3,054	3,054	3,130

CG is Cockroft-Gault; MDRD is Modified Diet for Renal Disease; CKD-EPI is Chronic Kidney Disease- Epidemiological Collaboration equations

The frequencies (95% CI) of renal impairment determined by an eGFR of <60ml/min using the Cockroft-Gault, MDRD and CKD-EPI formulae were 38.8% (37.1% - 40.5%), 13.7% (12.6% to 15.0%) and 13.9% (12.7% to 15.1%) respectively, while the frequencies of severe renal impairment with eGFR <30ml/min were 5.7% (4.9% to 6.6%), 3.1% (2.5% to 3.7%) and 3.1% (2.6% to 3.8%) respectively. Given that none of these formulae has been validated for use in HIV-infected patients, results of analysis of the risk factors for HIV-related renal impairment were conducted using all 3 formulae and those for Cockroft-Gault and the CKD-EPI are shown. On adjusted analysis, the risk factors associated with an eGFR of <60ml/min calculated using the Cockroft Gault formula were increasing age - increased risk of 108% for each 10 years older, male gender - 51% lower risk compared with females, CD4 count at baseline - a 5% lower risk for each 50 cells increase in baseline CD4 count and advanced WHO clinical stages of HIV disease - 35% higher risk for each stage higher. However, using the CKD-EPI, only increasing age was significantly associated with the risk of developing HIV-related renal-impairment (Table 3.7).

Severe renal impairment with eGFR <30ml/min calculated using Cockroft Gault was significantly associated with increasing age, low CD4 counts, advanced WHO clinical stages and having a diagnosis of systemic arterial hypertension at baseline. Using the CKD-EPI formula, the only factor associated significantly with severe renal impairment was a low CD4 count at baseline with marginal but non-significant associations between increasing age and WHO clinical stage (Table 3.8).

Table 3.7. Risk factors for estimated glomerular filtration rate <60ml/min.

Variable	eGFR by Cockroft-Gault formula				eGFR by CKD-EPI			
	Unadjusted OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value	Unadjusted OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value
Age /10 year higher	1.93 (1.77-2.11)	0.0000	2.08 (1.90-2.29)	0.0000	1.60 (1.44-1.79)	0.0000	1.59 (1.42-1.77)	0.0000
Male gender	0.68 (0.58-0.81)	0.0000	0.49 (0.40-0.58)	0.0000	0.90 (0.71-1.14)	0.39	-	-
CD4 /every 50 cells higher	0.97 (0.93-1.00)	0.07	0.95 (0.91-0.99)	0.016	0.98 (0.93-1.03)	0.48	-	-
WHO stage/ each stage higher	1.35 (1.22-1.50)	0.0000	1.35 (1.20-1.51)	0.0000	1.06 (0.92-1.23)	0.38	-	-
Hypertension	2.25 (1.28-3.95)	0.047	1.18 (0.62-2.24)	0.62	2.66 (1.45-4.90)	0.0017	1.32 (0.65-2.68)	0.44
Diabetes mellitus	2.03 (0.89-4.64)	0.09	1.28 (0.45-3.67)	0.64	3.91 (1.70-9.00)	0.003	2.05 (0.70-6.00)	0.19
Hypertension & Diabetes mellitus	7.79 (0.91-66.76)	0.06	3.95 (0.32-48.75)	0.28	12.93 (2.36-70.84)	0.003	3.81 (0.46-31.92)	0.22

Table 3.8. Risk factors for estimated glomerular filtration rate of <30ml/min.

Variable	eGFR by Cockroft-Gault formula				eGFR by CKD-EPI			
	Unadjusted OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value	Unadjusted OR (95%CI)	p-value	Adjusted OR (95%CI)	p-value
Age /10 year higher	1.65 (1.41-1.92)	0.0000	1.62 (1.38-1.90)	0.0000	1.23 (1.00-1.52)	0.05	1.21 (0.98-1.50)	0.08
Male gender	0.91 (0.64-1.30)	0.61	-	-	1.00 (0.64-1.59)	0.99	-	-
CD4 /every 50 cells higher	0.89 (0.82-0.97)	0.008	0.91 (0.83-0.99)	0.03	0.87 (0.78-0.98)	0.02	0.89 (0.78-1.00)	0.05
WHO stage/ 1 stage higher	1.61 (1.27-2.04)	0.0000	1.54 (1.20-1.97)	0.0006	1.41 (1.04-1.90)	0.03	1.32 (0.97-1.81)	0.08
Hypertension	3.20 (1.48-6.94)	0.003	2.52 (1.13-5.65)	0.02	2.67 (0.94-7.59)	0.06	2.20 (0.65-7.44)	0.21
Diabetes mellitus	1.59 (0.37-6.84)	0.53	-	-	1.36 (0.18-10.18)	0.77	-	-
Hypertension & Diabetes mellitus	-	-	-	-	6.29 (0.73-54.45)	0.09	2.40 (0.21-28.04)	0.49

HBV co-infection rates and hepatic transaminase concentrations in the cohort: 1,534 (38.0%) patients had HBV serology results available, out of which 233 were sero-positive giving an estimated prevalence of 15.2%. Baseline serum ALT and AST values were available for 3,702 (91.6%) and 3,745 (92.7%) patients respectively. The mean ± SEM concentrations of ALT and AST prior to initiation of therapy were 37.1 ± 0.60 IU/L and 50.2 ± 0.68 IU/L respectively. Although the mean ± SEM serum ALT concentrations in male patients were significantly higher than in females, namely 39.3 ± 1.06 IU/L vs 36.1 ± 0.73 IU/L, p=0.0135, their serum AST concentrations were not; 49.7 ± 1.03 vs 50.6 ± 0.88, p=0.5. The mean ± SEM concentrations of AST and ALT in HBV HIV co-infected patients (n=223) were significantly higher than in HIV+HBV- (n=1,202) patients: 59.7 ± 3.81 U/L vs 48.5 ± 1.07 U/L, p=0.0002 for AST concentrations and 43.9 ± 2.60 U/L vs 37.0 ± 0.92 U/L, p= 0.0043 for ALT values respectively. Overall, 3,205 (86.6%) had no derangements in serum ALT, often taken as a specific marker of hepatocyte damage compared with AST which has several other sources aside hepatocytes, 404 (10.9%) had grade 1, 73(2.0%) had grade 2, 14 (0.4%) had grade 3 and 5 (0.1%) had grade 4 derangements in serum ALT concentrations. Baseline factors associated with the odds of an elevated ALT on univariate analysis were male gender, increasing WHO stage of HIV disease, decreasing CD4 count and increasing age but not HBV co-infection. On multivariate analysis, factors that remained significantly associated with the risk of an elevated baseline ALT were male sex (35% increased odds compared with females), increasing WHO clinical stage of HIV disease (17% increased odds for each unit rise in WHO stage with stage 1 as reference stage) while increasing baseline CD4 cell count was associated with a decreased odds of an elevated ALT (15% decreased odds for each 50 cells rise in CD4 count) as shown in Table 3.9. When a similar analysis was conducted to evaluate risk factors associated with severe liver disease corresponding to grades 3 and 4 transaminitis, no significant associations were noted for the aforementioned risk factors (data not shown).

Table 3.9. Baseline risk factors associated with elevated ALT levels in HIV infected Ghanaians by univariate and multivariate logistic regression models.

Baseline characteristic	Unadjusted OR (95% CI)	p-value	Adjusted OR (95% CI)	p-value
Sex (male vs female)	1.38 (1.13-1.68)	0.0013	1.35 (1.09-1.68)	0.0062
WHO stage (per each stage rise)	1.29 (1.13-1.48)	0.0003	1.17 (1.01-1.35)	0.0321
CD4 T-cell count (per each 50 cells increase)	0.83 (0.79-0.88)	0.0000	0.85 (0.81-0.91)	0.0000
Age (per 10 year increase)	0.90 (0.81-0.99)	0.0418	0.93 (0.83-1.03)	0.1671
HBV co-infection (positive vs negative)	1.28 (0.88 –1.87)	0.1899		

Overall model fit for 5 baseline predictors for elevated serum ALT in a multivariable model: Chi-square= 59.46, df=4, p=0.0000.

Serum lipid profile of cohort: A limited number of patients had lipid profile assessed before initiation of cART. The median (IQR) concentrations of serum total cholesterol was 4.0 (3.2 – 4.8 mmol/l, n=688), serum low-density cholesterol (LDL-C) was 2.4 (1.7 – 3.2 mmol/l, n=458), serum high-density cholesterol (HDL-C) was 1.0 (0.8 – 1.4mmol/l, n=481) and serum triglyceride was 1.5 (1.1 – 2.1 mmol/l, n=722). 135 (20%) had hypocholesterolaemia, 503 (73%) had normal serum cholesterol concentrations while 50 (7%) had hypercholesterolaemia. During the course of HIV disease, a complex, dynamic and bi-directional interaction between progression of HIV disease and serum lipid profile has been reported with hypocholesterolemia characterising early stages of HIV disease and hypertriglyceridaemia associated with later stages[369 - 374]. An exploratory analysis was therefore performed to ascertain these observations in the present cohort. As noted above 135 out of 688 (20%) patients with available data had hypocholesterolaemia while 114 out of 722 (16%) had hypertriglyceridaemia. On univariate analysis, baseline factors significantly associated with hypocholesterolemia were WHO clinical stage, baseline CD4 T-cell count and BMI but not gender nor age. Furthermore on multivariate analyses, significant adjusted odds ratio (95% CI) for hypocholesterolemia were 1.69 (1.14 -2.51, p=0.009) for each increment in WHO clinical stage and 0.42 (0.28 – 0.64, p=0.0000) for each 5kg/m^2 increment in BMI. However when similar logistic regression analyses were performed to identify baseline risk factors for hypertriglyceridaemia, none of the variables - namely age, gender, WHO stage, CD4 count nor BMI - were significantly associated with hypertriglyceridaemia on univariate analysis. Thus in this cohort, risk of hypocholesterolaemia was strongly associated with progressive or late HIV disease and low body mass index. Additional analysis was carried out to identify factors associated with hypercholesterolaemia at baseline which was present in 7% of subjects. The only significant factor associated with risk for hypercholesterolemia identified on multivariable analysis was WHO clinical stage with an adjusted odds ratio (95% CI) of 2.16 (1.22 – 3.82, p=0.008) per each stage higher.

Demographic, clinical and laboratory features of patients according to NNRTI/PI

A descriptive comparison of baseline clinical and laboratory characteristics of patients according the either the NNRTI or PI base chosen to initiate cART is shown in Table 3.10. Briefly, 2,376 (58.8%) patients were started on efavirenz (EFV) based cART compared with 1,623 (40.2%) on nevirapine (NVP) based cART while only 40 (1.0%) were initiated on protease inhibitor based cART. Of the 40 patients who started a PI-based cART, due to HIV-2 mono- or HIV-1/2 dual infections, 23 were started on ritonavir-boosted lopinavir with the remaining 17 starting on nelfinavir. Two thousand one hundred and three (2,103) patients representing 52.1% of patients were started on an NRTI backbone of stavudine (d4T) and lamivudine (3TC) while 1,925 (47.7%) were initiated on zidovudine (AZT) plus lamivudine (3TC) with 11 (0.2%) patients commencing on other backbone NRTIs such as tenofovir (TDF) plus lamivudine (3TC)-5 patients, didanosine (ddI) plus abacavir (ABC)-4 patients, abacavir plus lamivudine-1 patient and didanosine plus stavudine-1 patient.

Significantly more females than males were initiated on NVP-based cART compared with EFV-based cART while those initiating EFV-based cART were significantly older than those on NVP-based cART. There were no significant differences in the clinical stage of patients at initiation of therapy with respect to the NNRTI or PI used. The mean ± SEM BMI (in kg/m^2) of patients commencing therapy EFV-based cART was 20.1 ±0.09 vs 20.5 ± 0.10 for those on NVP-based cART and 21.0±0.62 for those initiating a PI-based cART, p=0.0025 (ANOVA). The median (range) CD4 T-cell counts in patients initiating EFV-based cART of 127.5 (1-1,085/mm^3) was significantly lower than those of patients initiating NVP-based cART of 140.0 (0-676/mm^3) vs 186.0 (1-

1134) of those on PI-based cART, $p=0.0006$. No differences were observed in the median haemoglobin concentration between the 3 groups at baseline. However serum ALT and AST concentrations were significantly higher among patients initiating EFV-based cART vs those on PI-based cART and NVP-based cART in descending order. Again, patients initiating EFV-based cART had a significantly lower median (IQR) estimated glomerular filtration rate of 64.0 (47.0-83.0ml/min/1.73m^2) vs 66.5 (54.5 – 89.5ml/min/1.73m^2) for those on PI-based cART and 71.0 (54.0 – 89.0ml/min/1.73m^2) for those on NVP-based cART, $p<0.0001$. Finally, there were no significant differences in the proportions of patients with HBV co-infection among the 3 groups.

Table 3.10. Baseline demographic, clinical and laboratory characteristics of patients initiating cART in Kumasi, Ghana.

Characteristic	Efavirenz n=2,376	Nevirapine n=1,623	Protease inhibitors n=40	Total n= 4,039	p-value
Male : female	1,028: 1,348	248:1,375	11: 29	1,287: 2,752	<0.0001
Median (range) age	40 (14 – 77)	35 (15 – 75)	36 (25 – 65)	38 (14 – 77)	<0.0001
WHO clinical stage n (%)					0.09
1	165 (6.9)	106 (6.5)	2 (5.0)	273 (6.8)	
2	258 (10.9)	225 (13.9)	6 (15.0)	489 (12.1)	
3	1274 (53.6)	867 (53.4)	25 (62.5)	2166 (53.6)	
4	407 (17.1)	238 (14.7)	10 (10.0)	649 (16.1)	
No data	272 (11.4)	187 (11.5)	3 (7.5)	462 (11.4)	
Mean BMI ± SEM	20.1±0.09	20.5±0.10	21.0±0.62	20.3 ± 0.07	0.0025
<18.5 kg/m^2	868 (36.5)	543 (33.5)	11 (27.5)	1422 (35.2)	0.0047
18.5 – 24.5 kg/m^2	1145 (48.2)	804 (49.5)	20 (50.0)	1969 (48.8)	
>24.5 kg/m^2	291 (12.3)	246 (15.2)	6 (15.0)	543 (13.4)	
No data	72 (3.0)	30 (1.8)	3 (7.5)	105 (2.6)	
Median (range) CD4 count	127.5 (1 – 1085)	140.0 (0 – 676)	186.0 (1 – 1134)	134 (0 – 1134)	0.0006
<200 cells/ml	1684 (70.9)	1080 (66.5)	21 (52.5)	2785 (69.0)	<0.0001
200-350 cells/ml	611 (25.7)	495 (30.5)	12 (30.0)	1118 (27.7)	
>350 cells/ml	53 (2.2)	41 (2.5)	7 (17.5)	101 (2.5)	
No data	28 (1.2)	7 (0.5)	0 (0.0)	35 (0.9)	
Median (range) Haemoglobin conc. (g/dl)	10.2 (2.6 – 19.4)	10.2 (3.2 – 19.8)	10.9 (6.8 – 16.3)	10.2 (2.6 – 19.8)	0.07
Mean ± SEM ALT (U/L)	40.5 ± 0.87	32.0 ± 0.75	39.3 ± 6.64	37.1 ± 0.60	<0.0001
Mean± SEM AST (U/L)	53.6 ± 0.93	45.2 ± 0.95	51.0 ± 11.6	50.2 ± 0.68	<0.0001
Median (IQR) eGFR ml/min/1.73m^2 , n	64.0 (47.0 –83.0) n=1791	71.0 (54.0 – 89.0) n=1240	66.5 (54.5 – 89.5) n=32	66.0 (50.0 -86.0) n=3063	<0.0001
> 60ml/min	1028 (57.4)	849 (68.5)	21 (65.6)	1898 (62.0)	<0.0001
30 – 59 ml/min	638 (35.6)	345 (27.8)	11 (34.4)	994 (32.5)	
15 – 29 ml/min	92 (5.1)	35 (2.8)	0 (0.0)	127 (4.1)	
<15ml/min	33 (1.8)	11 (0.9)	0 (0.0)	44 (1.4)	
HBV co-infection Positive/Negative (%)	143/761 18.8%	87/527 16.5%	3/13 23.1%	233/1301 17.9%	>0.05
NRTI backbone					
AZT + 3TC	1083 (45.6)	819 (50.5)	23 (57.5)	1925 (47.7)	<0.0001
d4T + 3TC	1286 (54.1)	804 (49.5)	13 (32.5)	2103 (52.1)	
Others	7 (0.3)		4 (10.0)	11 (0.2)	

Follow-up and vital status of patients at time of closing data for analysis

Ten thousand five hundred (10,500) patents with HIV infection enrolled at the clinic to receive care from January 2004 to 31st December, 2010. It is notable that the number of cases enrolling for care dipped between 2009 and 2010 because the clinic stopped enrolment of new cases due to increasing numbers of patients who were overwhelming limited number of care givers in the clinic. Patients who enrolled in the year 2011 were excluded from the present analysis because I wanted to examine patients who had had opportunity to receive at least 12 months of ART. Data was closed for analysis on the 31st of December 2011. Four thousand and thirty-nine (4,039) patients representing 38.8% of patients enrolled initiated cART, the yearly breakdown shown in Table 3.11. This figure may be an underestimation because some folders were missing and it is uncertain whether these missing folders were for patients who initiated cART or not. Although no further analysis was performed for the vast majority of patients who did not start cART (62% of registered patients) at the time of data analysis, most of these patients never returned back to the clinic after initial registration and were lost to follow up.

The recommended CD4 cut-off for initiation of ART was 200 cells/mm^3 between 2003 to 2007 and this threshold was increased to 350 cells/mm^3 from 2008 to date. Remarkably, there have been no corresponding differences in the median CD4 counts over the years of observation with the median yearly CD4 counts at initiation of ART ranging between the lowest of 124 cells/mm^3 in 2007 to 149 cells/mm^3 in 2010.

Table 3.11. Enrolment, characteristics, follow-up and vital status of patients initiating cART according to year of enrolment.

Year / Characteristic	2004	2005	2006	2007	2008	2009	2010	TOTAL	p-value
No. enrolled for ART	1,700	2,020	1,819	1,782	1,738	636	705	10,400	
No. starting ART	769	695	819	658	590	272	236	4039	
% starting ART	45.2	34.4	45.0	36.9	33.9	42.8	33.5	38.8	
WHO Clinical stage, n (%)§									<0.0001
1	52 (7%)	33 (5%)	43 (5%)	47 (7%)	53 (9%)	28 (10%)	16 (7%)	273 (7%)	
2	109 (14%)	80 (12%)	113 (14%)	70 (11%)	63 (11%)	25 (9%)	29 (12%)	489 (12%)	
3	460 (60%)	402 (58%)	420 (51%)	355 (54%)	280 (47%)	134 (49%)	115 (49%)	2166 (54%)	
4	126 (16%)	121 (17%)	145 (18%)	98 (15%)	80 (14%)	38 (14%)	41 (17%)	649 (16%)	
No data	22 (3%)	59 (8%)	98 (12%)	88 (13%)	114 (19%)	47 (16%)	35 (15%)	452 (11%)	
Median (IQR) CD4 count	136 (51 - 213)	136 (65 - 211)	133 (51 - 212)	124 (33 – 220)	131 (48 - 228)	128 (41-251)	149 (57 – 231)	134 (51 – 218)	0.23
Vital status									
Alive*	503(65%)	426(61%)	541(66%)	467(71%)	438(74%)	186(68%)	187(79%)	2748(68%)	<0.0001
Dead	35(5%)	40(6%)	50(6%)	104(16%)	63(11%)	14(5%)	18 (8%)	324(8%)	
Lost	231(30%)	229(33%)	228(28%)	87 (13%)	89(15%)	72(27%)	31(13%)	967(24%)	
Person follow up years	3565.1	2531.8	2158.7	1393.8	936.4	455.2	195.8	11236.8	

* And accessing the clinic, § % of patients initiating cART.

There were 11,236.8 person years of follow-up on ART with 2,748 patients (68%) still alive, 967 (24%) lost to follow up and 324 (8%) deaths at the time of closing data for this analysis. The median (IQR) follow up time per patient overall was 30 (12 – 54) months with a range from 0 to 90 months. The median (IQR) time of follow up of patients still alive, lost to follow up and dead at time of censoring were 42 (24-60) months, 6 (2-18) months and 2 (2-6) months respectively, $p<0.0001$. Table 3.12 shows a summary of a comparison of baseline characteristics of patients according to their vital status at censoring. It is evident that there are significant differences in clinical stage, body weight or BMI, CD4 T-cell counts, haemoglobin concentration, serum alanine transaminase, aspartate transaminase, total cholesterol, triglyceride and estimated glomerular filtration rate between survivors, lost to follow up and cases that died. The only baseline characteristic that did not significantly differ between the three groups was the median age. Compared to patients who died, patients who survived had lower clinical stage of disease, had higher mean body weight (in kg), had higher body mass index, had higher baseline median (IQR) CD4 counts, had higher baseline haemoglobin concentration, higher estimated glomerular filtration rate and higher serum total cholesterol, but had lower serum triglyceride concentration as well as lower AST and ALT concentrations as shown in Table 3.12.

Table 3.12. Comparison of baseline characteristics according to vital status of patients at close of data for analysis.

Characteristic	Alive* n=2748	Dead n=324	Lost n=967	Total n=4039	p-value
Male : female	788 : 1960	136 : 188	363 : 604	1287 : 2752	<0.0001
Median (IQR) age	38 (32 – 45)	38 (32 – 46)	38 (32 – 45)	38 (32 – 45)	0.91
Median (IQR) time of follow up in months	42 (24 – 60)	2 (2 – 6)	6 (2 – 18)	30 (12 – 54)	<0.0001
WHO stage					<0.0001
1	226	11	36	273	
2	377	14	98	489	
3	1425	178	563	2166	
4	364	99	186	649	
No data	356	22	84	462	
Mean ± SEM weight (kg)	53.2 ± 0.22	47.1 ± 0.60	50.5 ± 0.36	52.1 ± 0.18	<0.0001
Median (IQR) BMI (kg/m^2)	20.3 (18.0 –23.1)	17.6 (15.6-20.0)	18.8 (16.9-21.8)	19.8 (17.5-22.7)	<0.0001
Median (IQR) CD4 count	151 (68 – 229)	58 (13 – 146)	105 (34 – 193)	134 (51 - 218)	<0.0001
Median (IQR) haemoglobin g/dl	10.4 (9.0 – 11.6)	9.4 (7.9 – 10.8)	9.7 (8.4 – 11.2)	10.2 (8.8 – 11.5)	<0.0001
Mean ± SEM AST IU/l	48.8 ± 0.81	61.5 ± 3.22	50.8 ± 1.29	50.2 ± 0.68	<0.0001
Mean ± SEM ALT IU/l	36.3 ± 0.71	47.6 ± 3.29	35.7 ± 1.00	37.1 ± 0.60	<0.0001
Median (IQR) eGFR ml/min	67.8 (52.3-87.8)	54.0 (38.5-75.4)	65.3 (47.7-85.0)	66.4 (50.1-86.1)	<0.0001
Median (IQR) total cholesterol mmol/l	4.1 (3.3-4.9)	3.3 (2.6-4.6)	3.6 (3.0-4.5)	4.0 (3.2-4.8)	<0.0001
Median (IQR) serum triglycerides mmol/l	1.4 (1.1-2.0)	1.7 (1.2-2.3)	1.6 (1.2-2.3)	1.5 (1.1-2.1)	0.022

* And accessing the clinic

The high number of patients lost to follow up prompted a sub-study to evaluate the vital status of this category of patients. Given the wide geographic distribution of patients within the clinic, this survey was limited only to patients who gave telephone contacts. Two hundred and ten (210) patients out of 967 (22%) had telephone contacts for this survey. 51% of these contacts could not be reached by their telephone and therefore could not be analysed further. Overall, 39 (19%) of patients lost to follow up were still alive, the majority still on cART, 64 (30%) had died from AIDS related illnesses and 107 (51%) could not be contacted and therefore their vital status could not be ascertained as shown in Table 3.13.

Table 3.13. Vital status from a telephone survey of contacts of 210 patients lost to follow-up.

Vital status Year of registration	Alive	Dead	"Lost"	Total
2004	4	5	10	19
2005	4	15	22	41
2006	3	7	10	20
2007	3	12	15	30
2008	1	1	3	5
2009	19	10	27	56
2010	5	14	20	39
Total	39 (19%)	64 (30%)	107 (51%)	210

"Lost" refers to no response from contact.

Discussion

Although antiretroviral therapy has significantly improved morbidity and mortality outcomes in Africa, it has become important to evaluate the long-term effectiveness of the recommended first line cART in routine use. Important lessons drawn from these evaluations hopefully will point us towards the direction of rational use of antiretroviral medications in a region which has severe constraints on resources but is the home of 60% of the world's population of those living with HIV[1]. It is well-known that baseline factors such as AIDS diagnosis, severe anaemia, low body mass index and reduced CD4 counts are few amongst several other important determinants of both short-term and long-term outcomes of treatment in both developed and developing countries[375-381]. As a prelude to three subsequent chapters that examine rates of ART-related toxicity (Chapter 5) and clinical/immunological outcomes of ART (Chapters 4 and 6), this chapter focuses on a descriptive cross-sectional analysis of baseline characteristics of a cohort of HIV-infected patients who started ART in Kumasi, Ghana.

Since its inception in 2004 to December 31st 2010, nearly 10,500 patients enrolled for HIV care of which only 4,039 (38%) patients were started on cART. Thus the significant majority of patients accessing ART care (62%) never started the journey and most of these patients were lost to follow up from their first visit (personal observations from inspecting their folders). This pre-treatment attrition rate recorded is very high compared with reported rates of between 14.0% to 32.1% from other ART programmes in Africa such as South Africa[382], Malawi[383], Uganda[384] and the Gambia[385]. Although factors for this high pre-treatment attrition rate were not explored in this study, the commonly cited reasons for pre-treatment attrition are loss-to-follow ups and deaths, with the latter strongly influenced by low CD4 counts and advanced clinical disease[386]. There is the need to explore the factors associated with pre-treatment attrition among HIV care seekers in the Ghanaian ART programme.

The clinical and demographic characteristics of the patients who started cART were no different from those reported from similar other cohorts in Africa. Patients were young, median age of 38 years, with a preponderance of females and had advanced clinical disease- nearly 80% had WHO clinical stages 3 and 4 disease. The common AIDS defining conditions identified among patients initiating cART in our programme were severe weight loss, oro-oesophageal candidiasis and tuberculosis. The poor outcomes of starting cART among patients who were severely immunocompromised prompted a policy change in 2007 by the national programme in conformity with the WHO guidelines for the CD4 threshold at initiating cART to be lifted from below 200 cells/mm^3 to 350 cells/mm^3. However as Table 3.11 shows, after 7 years of the programme in Ghana, the starting CD4 counts, proportions with severe HIV disease initiating therapy and pre-treatment attrition rates have not changed significantly. Also, more females initiated cART than male, which has also been observed in several cohorts across Africa. One likely reason is the expansion of HIV testing for pregnant women in antenatal care through PMTCT programs leading to identification of HIV among asymptomatic women[387, 388]. The weak health care infrastructure and the lack of integration of health pathways have been identified as the major hurdles to timely initiation of cART[389-391]. Thus there is the urgent need to refocus on a strengthened delivery system that will minimize delays in initiation of ART and help mitigate the clinical, social and economic barriers to accessing ART among eligible HIV infected patients. Clearly, measures to increase uptake of HIV testing in healthcare facilities and in other settings such as in schools, workplaces, and in churches may increase early diagnosis of HIV to engender early initiation of therapy especially in men.

Combination antiretroviral therapy used in initiating therapy in many programmatic settings comprises of a backbone of two thymidine nucleoside reverse transcriptase inhibitors namely zidovudine or stavudine plus lamivudine with one non-nucleoside reverse transcriptase inhibitor of either nevirapine or efavirenz. In our programme, a significantly higher proportion of patients started efavirenz- compared to nevirapine-based cART and the likely reason for this is that nevirapine was contraindicated in many patients who were on anti-tuberculous medications when cART was initiated. This together with the fact that significantly more males were started on efavirenz compared with females could explain why the CD4 counts of patients initiating efavirenz-based cART was significantly lower than those starting nevirapine-based cART (Table 3.10). Whether these factors would affect the effectiveness of efavirenz compared with nevirapine is explored in chapter 6 of this dissertation.

From the metabolic point of view, the integrity of the renal and liver functions are important determinants for the initial selection of cART as well as the predisposition to potential adverse events. Whereas NRTIs are metabolised and excreted principally by the kidneys, the NNRTIs are dependent on processing by hepatic metabolic pathways (reviewed under section 1.2.4.2). Thus consideration should be given to the incidence and risk factors of HIV-associated kidney disease and liver dysfunction in these settings.

Using the Cockroft Gault formula to determine the eGFR, we found 38.8% of patients initiating therapy had renal dysfunction with an eGFR< 60ml/min. This is in agreement with the prevalence of 38.0% among a Nigerian[392] cohort but higher than 11.5%, 8.7%, 7.0% and 4.9% among Kenyan[393], Zambian[394], Ugandan[395], Zimbabwean[395] and Burundian[396] cohorts of naïve HIV-infected patients respectively. The risk factors identified among Ghanaian patients were female gender, increasing age, advanced stage of HIV disease and decreased body mass index. Among the Nigerian cohort[392], patients with renal dysfunction defined by proteinuria as well as raised serum creatinine were more likely to be older, have a lower BMI and lower CD4 counts, findings which are comparable with those from the present study. Various authors have shown that high viral load and low CD4+ T-cell lymphocyte counts[397-402] are pathogenically associated with an increased risk of developing an HIV-related CKD, reflecting the increased incidence of CKD in individuals with poorly controlled HIV. These observations underline the fact that renal impairment is an indicator of progressive HIV disease, hence routine screening of all patients enrolled in cART programmes across SSA should be encouraged and treatment initiated among those with renal impairment. Although diabetes mellitus and hypertension are known to be associated with renal impairment, patients with these co-morbidities were included in analysis to determine the association between these 2 common causes of renal insufficiency in a cohort known to have HIV-infection and as shown in Table 3.8, hypertension was associated with the higher risk of severe renal insufficiency.

There is a wide spectra of HIV-related kidney diseases of which the commonest histological entity is called HIV-associated nephropathy which is commoner with advanced HIV disease and has an increased predilection for African American patients[402 - 404]. Among African Americans, genetic studies have recently identified a higher frequency of recessive polymorphisms in the MYH9 gene and the neighbouring

APOL1 gene both located on chromosome 22q13 locus which predisposes to an increased risk for HIV-1-associated nephropathy and idiopathic focal segmental glomerulosclerosis[405 - 408].

A limitation of using creatinine assessments without analysis of proteinuria is that there is a tendency to underestimate the prevalence of chronic renal failure and tubular dysfunction which may not be detected by creatinine measurements. Although none of the 3 formulae used to calculate eGFR have been validated for HIV-infected patients, the National Kidney Foundation guidelines generally recommends creatinine-based GFR estimation and is preferred to serum creatinine alone, which is accepted to have a lower sensitivity for detecting renal impairment[409, 410]. Among these three equations used to determine eGFR, both the MDRD and CKD-EPI were shown in a large Ghanaian predominantly Ashanti adult population to over-estimate the creatinine clearance by 18.2 and 19.0 ml/min/1.73 m^2 respectively whereas the mean GFR estimated using the Cockroft-Gault equation was 9.4 ml/min/1.73 m^2 lower[411]. The findings from the Ghanaian study[411] which was conducted in the same geographical location where most of patients in this cohort resided, suggest that in lean African populations, eGFR determined by Cockroft and Gault formula may be closer to creatinine clearance since it takes into account the body weight of the subject while the MDRD and CKD-EPI does not. Hence in HIV-infected patients with advanced disease evidenced by low body mass indices, the Cockroft-Gault equation may approximate the creatinine clearance more closely. Certainly further studies are needed to examine which of these formulae estimates GFRs more closely, as well as longitudinal studies of changes in GFR on antiretroviral therapy and the impact of renal dysfunction on risk for mortality.

A hepatitis B virus co-infection rate of 15.2% was found among 38% of the cohort who had HBV serology performed. Data in agreement with ours from other West African countries have reported co-infection rates of 12% to 17% (Senegal[412], Burkina Faso[413], Nigeria [414] and Ivory Coast [415]) and 2% to 20% in East and South Africa (Kenya [416], Uganda[417] Rwanda[417], Malawi[418] and South Africa[419]). The routine testing of HBV among HIV patients is important for three principal reasons. First, HIV infection is well-known to have a promoting effect on HBV replication and progression of hepatic damage[420-423]. Second, in settings where first line therapy usually uses lamivudine in combination with either zidovudine or stavudine and nevirapine or efavirenz, patients with HBV/HIV co-infection are essentially on lamivudine monotherapy (instead of the preferred dual therapy) for HBV with the attendant increased risk of HBV drug resistance and progression of liver disease[424]. Until recently tenofovir was often reserved for use after failure of the initial regimen. However, in our setting tenofovir is the only other antiretroviral with activity against HBV which could be used in combination with lamivudine as part of the preferred first line therapy for patients with HBV co-infection. Third, there is a known significantly increased risk of hepatotoxicity in HBV co-infected patients who initiate NNRTIs with risk particularly pronounced with the use of nevirapine. Although a clear association was not demonstrated between baseline concentrations of liver enzymes and hepatitis B sero-positivity, it has been clearly demonstrated that levels of liver enzymes are not very sensitive predictors of extent of hepatic damage in chronic HBV infections. In Chapter 5, further analysis is presented on the impact of HBV co-infection and the risk for hepatotoxicity on cART in this cohort.

This cohort, that has been established in Kumasi for this present analysis and for future prospective studies, is reasonably large with long follow-up periods for most patients, median follow up of 30 months (range of 0 to 7 years) and has 11,236.8 person years of follow-up treatment experience on ART in a programmatic setting at censoring. At the close of 31st December 2011, 2,748 patients (68%) were still alive and accessing the clinic, 967 (24%) were lost to follow up and 324 (8%) had died. In a systematic review by Rosen and colleagues, retention in ART programmes in SSA ranged between 39.2% to 90.3% over a follow-up period of 6 to 46 months in 33 patient cohorts from 13 countries[425]. The risk factors and predictors of deaths and loss-to-follow up are further examined in Chapter 4. However, it suffices to say at this point that a majority of those lost from the programme may have died. In similar cohorts, it has been shown that significant majority of patients lost to follow up in the first year of initiating cART had died. In support of this, the telephone survey from the present study showed that about half of 210 patients were contactable, of which over 60% had died. This provides some basis for the speculation that most patients who are lost-to-follow up from ART programmes may have died.

In conclusion, there is a high frequency of cART pre-treatment attrition among HIV-infected patients accessing cART in a busy HIV clinic in Kumasi. Those initiating cART are profoundly immunosuppressed and have advanced clinical stages of HIV infection with a high frequency of renal impairment. A higher proportion of patients commenced a predominantly NNRTI-based cART of either efavirenz or nevirapine on a dual backbone of either stavudine plus lamivudine or zidovudine plus lamivudine. Attrition from the programme were comparable to those from other ART programmes across SSA and factors influencing attrition and long-term responses are evaluated in the next chapter.

CHAPTER FOUR

4.0 The long-term immunological and clinical effectiveness of combination antiretroviral therapy among Ghanaian HIV-infected patients.

4.1 Introduction

Combination anti-retroviral therapy (cART) for the long-term management of HIV infection is administered to people living with HIV/AIDS primarily to achieve long-term suppression of virological replication and to maintain CD4 cell counts at a level that reduces the risk of morbidity and mortality. Indeed there are many cohort studies in both the developing and the developed countries that have compared the short- to medium-term effectiveness of different cART regimens. It is encouraging that the effectiveness of cART in developing countries in Sub-Saharan Africa has been reported to be similar, and often superior in clinical and immunologic outcomes when compared with those from the developed countries[426-433]. Whether these initial favourable immunological and clinical responses are sustained over the long-term remains to be determined.

Deaths in the era of cART has largely been due to AIDS-defining clinical events in many such reports from developing countries but the dynamics of mortality is believed to be changing in the industrialised countries with Non-AIDS defining clinical events assuming importance as causes of death as patients live longer on these potent antiretroviral medications[434-436]. Non-AIDS defining events are classified as cardiovascular, renal, hepatic-related or non-AIDS-defining malignancies that are likely to have an impact on morbidity and mortality[437]. One report from Botswana intimated that the age-standardised incidence rates of Non-AIDS defining events were comparable to those in the United States[438]. However, the spectra of disease entities included in this definition is debated[436] and does not capture infectious disorders such as malaria which is a common cause of morbidity among patients in Sub-Saharan Africa. In the present analysis, the frequencies and incidence rates of NADEs were classified using established Division of AIDS (DAIDS) tables for Grading Severity of Adult Adverse Experiences (NADEs)[358, 359]. The incidence rates of infectious diseases and other medical co-morbidities that cause significant morbidity but which does not meet the criteria of AIDS-defining are presented as ‘medical co-morbidity on cART’ separately from the conventional NADEs.

Attrition from ART programmes are withdrawals from programmes due either to deaths, loss to follow ups or transfer to another treatment site. Given that death is such an important treatment outcome measure, an analysis was performed to determine the risk factors and the causes for those in whom it was known. Furthermore due to the high prevalence of renal impairment at baseline among this cohort, an analysis was performed to determine whether renal impairment was independently associated with death. However any analysis of death as an outcome measure of cART is complicated by loss-to-follow up. To address this issue of loss-to-follow up, a survey was conducted and has been reported in chapter 3 among contacts of a sub-group of patients lost-to-follow up which suggested that most of these patients might have died. Furthermore, analysis of baseline predictors of loss-to-follow up was conducted to determine whether they were similar to among patients known to have died. This chapter presents an analytical overview of the major immunological and clinical outcomes on long-term cART among this cohort of over 4,000 Ghanaian HIV patients initiating cART within 2004 to 2010. The aims of this chapter are to describe the trends in CD4 and body mass index changes on cART as well as the risk factors and incidence rates for AIDS, non-AIDS clinical events, adherence, mortality and loss to follow up.

4.2 METHODS

Refer to chapter 2 section 4.

4.3 RESULTS

Baseline demographics and laboratory characteristics of study participants

Baseline demographic and laboratory characteristics of the study participants are described in chapter 3.

Changes in CD4 counts with cART

The median (IQR) CD4 count at baseline of 133 (50 – 218) increased to 314 (204 – 429) within 6 months of initiation of ART, p<0.0001. This initial increment was sustained at 12 months with a median (IQR) CD4 at 12 months of 355 (244 – 487), with further increases during follow up to month 90 for patients who remained on cART and were accessing the clinic as shown in Figure 4.1.

Immune reconstitution inflammatory disorders: The restitution of the CD4 T-cell counts on cART may be accompanied by the occurrence of the well-known Immune reconstitution inflammatory response syndrome. In this cohort, there were 45 documented cases of IRIS with an overall incidence rate of 4.00 (2.92-5.36) events/1000 person years under follow up. Tuberculosis-associated IRIS was the commonest and manifested clinically as new pulmonary infiltrations (n=16), lymphadenitis (n=2) and pleural effusions (n=2) over a median of 2 months (range of 2 to 12 months). Others included herpes zoster (n=13) and cerebral toxoplasmosis (n=9) as shown in Table 4.1. The median age of patients experiencing IRIS was 38 years, 33

(73%) were females and the median CD4+ T-cell counts at initiation of cART was 73 (range, 2 to 314 cells/mm^3). Combinations of antiretrovirals initiated by these patients who subsequently developed IRIS comprised of a backbone of AZT plus 3TC (n=20) and D4T plus 3TC (n=25) on an NNRTI-base of either nevirapine (n=22) or efavirenz (n=23). At the close of data for analysis, 27 (60%) patients who developed IRIS were alive, 6 (13%) patients died and 12 (27%) were lost-to-follow up.

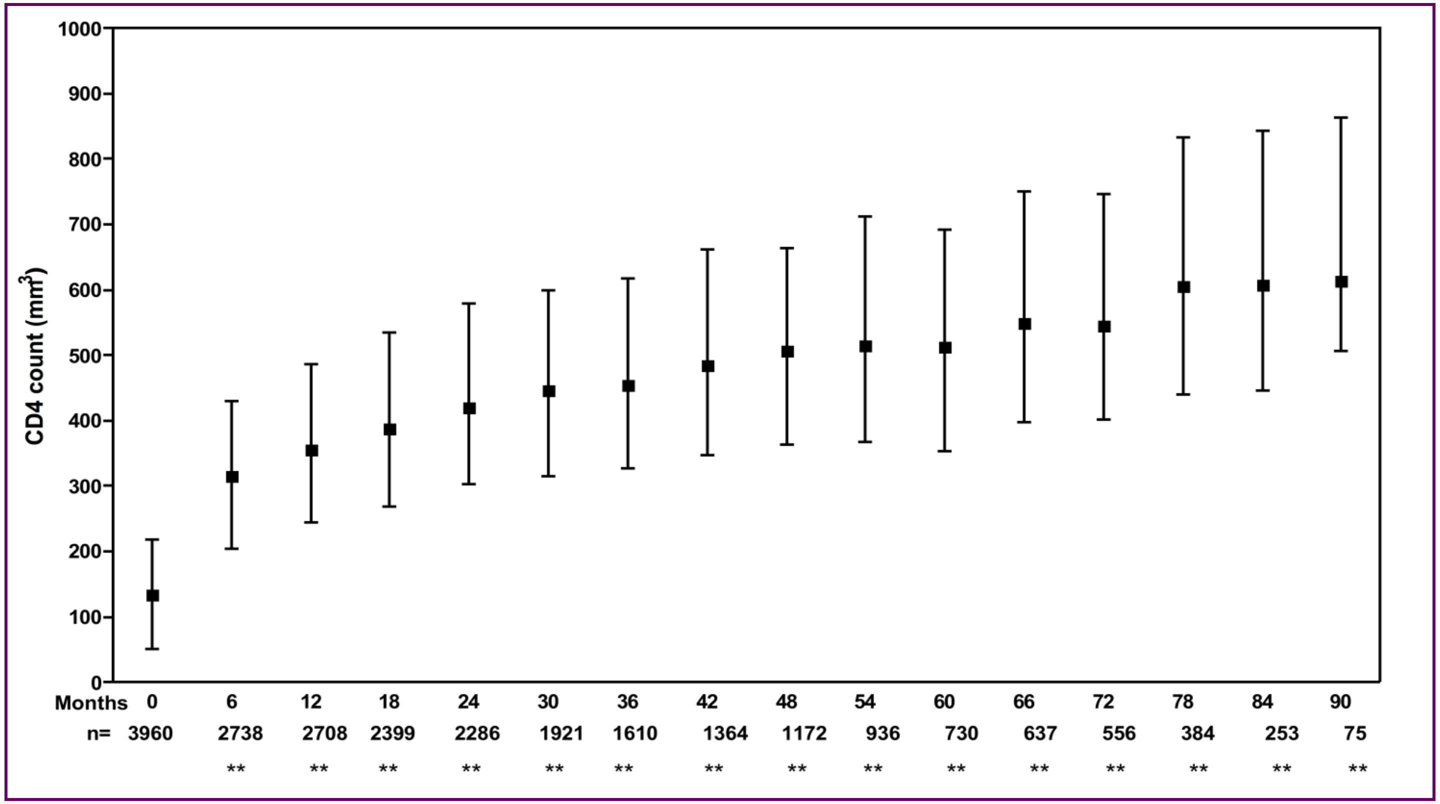


Figure 4.1. Changes in CD4 T-cell counts on cART. Each full square box represents the median, horizontal lines represent the Interquartile range and double asterixes indicate statistically significant difference obtained by pair-wise comparison of median with month 0 using the Mann-Whitney's U-test with $p<0.0001$ representing a statistically significant difference.

Table 4.1. Frequencies and rates of specific immune reconstitution inflammatory disorders among Ghanaian HIV-infected patients on cART.

Condition	Frequency	Rate/1000 person years follow up (95% CI)
Tuberculosis-pulmonary	16	1.42 (0.81 – 2.31)
Herpes zoster	13	1.16 (0.62 – 1.98)
Cerebral toxoplasmosis	9	0.80 (0.37 - 1.52)
CMV retinitis	2	0.18 (0.02 – 0.64)
Tuberculous lymphadenitis	2	0.18 (0.02 – 0.64)
Tuberculous pleural effusion	2	0.18 (0.02 – 0.64)
Cryptococcal meningitis	1	0.09 (0.00 – 0.50)
Total	45	4.00 (2.92 – 5.36)

Incidence and risk factors for immunological failure: Although robust CD4 T-cell recovery was noted among most patients on cART over the long-term, CD4 counts for others failed to increase above 100 cells/mm^3 after one year of cART or declined by more than 50% of its peak value thus fulfilling the WHO criteria for immunological failure. There were 407 immunological failures over the 11,236.8 person years of follow up giving a crude event rate of 3.62 (3.28-3.99) per 100 person years. The median (range) time to occurrence of immunological failure on first line ART was 24 (12 – 78 months). Factors significantly associated with the risk of immunological failure on multivariable Cox proportional hazards analysis were male sex with an adjusted HR of 1.85 (95% CI of 1.47-2.33, $p<0.0001$), initiating cART below 40 years of age with an adjusted 1.25(1.01-1.55), $p<0.04$ and baseline CD4 strata below 200 cells/mm^3 with an adjusted HR of 3.62 (95% CI of 2.59-5.07, $p<0.0001$). Of note neither the NRTI backbone nor the NNRTI used to initiate cART was significantly associated with the risk for immunological failure as shown in Table 4.2.

Table 4.2. Univariate and multivariate analysis of factors associated with risk of developing immunological failure on first line ART.

Variable	Patient follow-up time (years)	Number of events	Crude event rate (/100 person years)	Univariate hazard ratio (95% CI)	p-value	Adjusted hazard ratio (95% CI)	p-value
Sex							
Male	3467.2	167	4.82 (4.11-5.60)	1.64(1.34-1.99)	<0.0001	1.85(1.47-2.33)	<0.0001
Female	7769.6	240	3.09 (2.71-3.51)	1.00		1.00	
Age							
<40 years	6082.0	234	3.85 (3.37-4.37)	1.21 (3.37-4.37)	0.055	1.25(1.01-1.55)	0.04
≥ 40 years	5161.3	169	3.27 (2.80-3.81)	1.00		1.00	
WHO stage							
3 or 4	7803.0	295	3.78(3.36-4.24)	1.19 (0.92-1.55)	0.18	-	-
1 or 2	2370.3	72	3.04(2.38-3.83)	1.00			
Baseline CD4 strata							
<200	7706.4	359	4.66 (4.19-5.17)	1.16(1.08-1.24)	<0.0001	3.62(2.59-5.07)	<0.0001
>200	3461.8	45	1.30 (0.95-1.74)	1.00		1.00	
NRTI backbone							
D4T plus 3TC	5276.4	192	3.64 (3.14-4.19)	0.95 (0.78-1.15)	0.59	-	-
AZT plus 3TC	5939.8	214	3.60 (3.13-4.12)	1.00			
NNRTI							
Efavirenz	6119.9	216	3.53(3.07-4.03)	0.92 (0.75-1.12)	0.39	-	-
Nevirapine	5012.5	187	3.73(3.22-4.31)	1.00			

Clinical events of cART

Changes in BMI on cART: The median (IQR) body mass index at baseline was 19.8 (17.5 – 22.7 kg/m^2) and increased significantly on ART to 20.7 (18.3 – 23.5 kg/m^2) p<0.0001 at month 2 with further increase at 12 months to 23.2 (20.8 – 26.2 kg/m^2), plateauing between months 36 through 60 to month 90 at 23.5 (20.9 – 26.7kg/m^2) at 36 months, 23.8 (20.9 – 27.5 kg/m^2) at 60 months and 24.2 (20.7 – 28.5 kg/m^2) at 90 months with p<0.0001 at all time points compared with baseline as shown in Figure 4.2.

Non-AIDS defining clinical events: There were 41 Non-AIDS defining events fulfilling the criteria of the DAIDS definition of NADEs with the commonest recorded events being hepatic disorders (n=20), cardiovascular events (n=10), new onset severe renal impairment with eGFR below 30ml/min (n=6) and hepatocellular carcinoma (n=4) and oesophageal carcinoma (n=1) were the only non-AIDS defining malignancies recorded as shown in Table 4.3. The overall crude NADEs incidence rate (95% CI) was 3.65 (2.62 - 4.95) per 1000 person years.

Medical co-morbidity on cART: By far, the largest majority of morbidity was of infectious causes of which 2,377 events were documented and presented in Table 4.4 with malaria being the commonest event. This is followed by upper and lower respiratory tract infections (n=666) such as community-acquired pneumonia (n=274), a range of dermatological infections (n=364) such as furunculosis (n=116), herpes zoster (n=56) and tinea corporis (n=26) and then various gastroenteritides and urinary tract infections. Included in the category of miscellaneous events were dyspeptic disorders such as gastro-oesophageal reflux disease and duodenal ulcers; others include post-herpetic neuralgia and previously undiagnosed seizure disorders on cART with no intracranial masses on CT scan of the brain.

Of the non-infectious medical comorbidities recorded, ninety-four (94) and 9 patients developed systemic arterial hypertension and type 2 diabetes mellitus respectively while on cART.

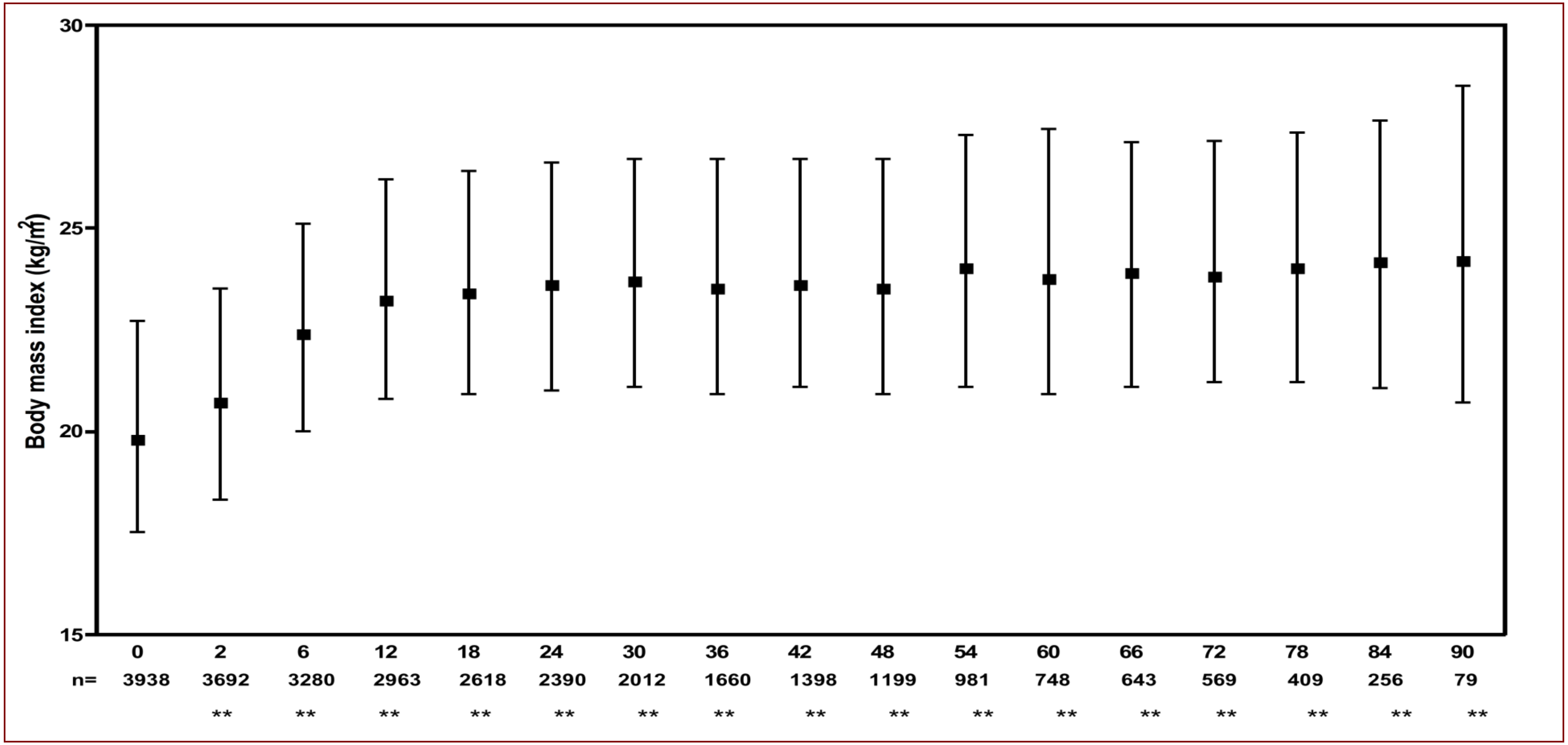


Figure 4.2. Changes in body mass index of patients on cART over time. Each closed box represents the median, horizontal lines represent the Interquartile range and ** shows median BMI compared with baseline shows significant statistical difference of <0.0001.

Table 4.3 Number and rates of Non-AIDS defining events on first line ART among Ghanaians.

Condition	Number of events	Rate/1000 person years follow up (95% CI)
Hepatic disorders		
Chronic liver disease	13	1.16 (0.62 – 1.98)
Hepatitis B viral infection flares	7	0.62 (0.25 – 1.28)
Cardiovascular events		
Stroke	8	0.71 (0.31 – 1.40)
Congestive cardiac failure	2	0.18 (0.02 – 0.64)
Renal disorders		
Stage 4 or 5 renal impairment	6	0.53 (0.19-1.16)
Non-AIDS malignancies		
Hepatocellular carcinoma	4	0.36 (0.10 – 0.91)
Oesophageal carcinoma	1	0.09 (0.00 – 0.05)
Total	41	3.64 (2.61 – 4.95)

Table 4.4. Frequencies and rates of other medical morbidity on cART among Ghanaians

Condition	Number of events	Rate/1000 person years follow up (95% CI)
Non-AIDS defining infectious disorders		
Malaria	746	66.39 (61.71 – 71.32)
Upper and lower respiratory infections	666	59.27 (54.85 – 63.95)
Dermatological infections	364	32.39 (29.15-35.90)
Gastroenteritis	314	27.94 (24.94-31.21)
Urinary infections	151	13.43 (11.38-15.76)
Ear nose and throat infections	102	9.08 (7.40 - 11.02)
Sepsis	29	2.58 (1.73 – 3.71)
Bacterial meningitis	5	
Miscellaneous disorders		
Dyspeptic disorders	73	6.50 (5.09 – 8.17)
Post-herpetic neuralgia	52	4.63 (3.46 – 6.07)
Seizure disorders	24	2.14 (1.37 – 3.18)
Paraparesis (unknown cause)	10	0.88 (0.43 – 1.64)
Hemiparesis (unknown cause)	9	0.80 (0.37 – 1.52)
Monoparesis (unknown cause)	6	0.53 (0.19 – 1.16)
Proximal myopathy	3	0.27 (0.06 – 0.78)
Cardiovascular risk co-morbidity		
Systemic arterial hypertension	94	8.36 (6.76 – 10.24)
Diabetes mellitus	9	0.80 (0.37 – 1.52)

The causes and factors associated with incidence of AIDS-defining conditions on ART

Six hundred (600) patients experienced a reported number of 681 AIDS-defining events during follow up on ART with a crude (95% CI) event rate of 60.60 (56.14 – 65.33) per 1000 person years of follow up. 531 (88.5%) patients had 1 AIDS-defining event, 59 (9.8%) had 2 events, 8 (1.3%) had 3 events, 1 (0.2%) had 4 events and 1 (0.2%) had 5 events under follow up. The median (range) time to development of first AIDS-defining event was 6 (2-90 months). The frequencies of 19 AIDS-defining conditions recorded during follow-up on ART are presented in Table 4.5. The leading five AIDS-defining events that occurred while on ART were pulmonary tuberculosis, chronic diarrhoea, oesophageal candidiasis, recurrent pneumonias and oral candidiasis at rates (95% CI) of 15.93 (13.68 – 18.44), 13.79 (11.71 – 16.14), 6.67 (5.25 – 8.36), 5.16 (3.92 – 6.67) and 4.00 (2.92 – 5.36) per 1000 person-years respectively of follow-up on cART. Nine patients were documented to have had computed tomographic evidence of an intracranial space occupying lesions while on cART, but clinicians did not document the etiology of these mass lesions but for the purposes of the present study, they adjudged to be AIDS-defining events.

Table 4.5. The frequencies and rates of various AIDS-defining conditions among Ghanaian HIV-infected patients on first line cART.

Condition	Frequency	Rate/1000 person years follow up (95% CI)
Pulmonary tuberculosis	179	15.93 (13.68 – 18.44)
Chronic diarrhoea	155	13.79 (11.71 – 16.14)
Oesophageal candidiasis	75	6.67 (5.25 – 8.36)
Recurrent pneumonia	58	5.16 (3.92 – 6.67)
Oral candidiasis	45	4.00 (2.92 – 5.36)
Cerebral toxoplasmosis	38	3.38 (2.39 – 4.64)
Extrapulmonary tuberculosis	33	2.94 (2.02 – 4.12)
Kaposi sarcoma	31	2.76 (1.87 – 3.92)
CMV retinitis	17	1.51 (0.88 – 2.42)
HIV encephalopathy	16	1.42 (0.81 – 2.31)
Cryptococcal meningitis	12	1.07 (0.55 - 1.87)
Intracranial space occupying lesion*	9	0.80 (0.37 – 1.52)
Non-Hodgkin’s disease	5	0.44 (0.14 – 1.04)
HIV wasting syndrome	3	0.27 (0.05 – 0.78)
Pneumocystis jirovercii pneumonia	2	0.18 (0.02 – 0.64)
CNS lymphoma	1	0.09 (0.00 – 0.50)
Herpes oesophagitis	1	0.09 (0.00 – 0.50)
Invasive cervical carcinoma	1	0.09 (0.00 – 0.50)
Total	**681**	**60.60 (56.14 – 65.33)**

AIDS defining events were defined using WHO clinical criteria. * Causes of Intracranial space occupying lesion on CT scan were not documented but were presumed to be AIDS-defining events.

Table 4.6 shows results of univariate and multivariate analyses of predictive factors associated with risk of developing an AIDS defining illness after initiating cART. In this analysis, the outcome of interest was time to development of an AIDS defining event. Patients who were alive at the end of the study censoring or closing time set at 31st December, 2011 or were lost-to-follow up before this date without an AIDS-defining event were right-censored. Factors significantly associated with the risk of developing an AIDS-defining event on cART on adjusted analyses were low body mass index below 16kg/m^2, WHO clinical stages 3 or 4 at baseline and CD4 strata below 200 cells/mm^3 at initiation of therapy. Initiating a D4T plus 3TC backbone was marginally associated with an increased risk of developing an AIDS- defining event, adjusted HR of 1.08 (0.90-1.30), p=0.09.

Of the 600 patients who developed various AIDS-defining events on cART, 317 (52.8%) were alive as at time of closure of data but 153 (25.5%) were subsequently lost-to-follow-up and 130 (21.7%) subsequently died. The median (range, IQR) duration between the first AIDS-defining diagnosis and death was 0.25 months (0 to 72, 0 to 2 months respectively) and that for loss-to-follow up was 4 months (0 to 76, 0 to 12 months respectively). Patients who developed AIDS-defining event and were alive at the time of closure of data for analysis survived for a median of 34 months (range of 0 to 88 months, IQR of 18 to 52 months).

Table 4.6. Univariate and multivariate analysis of factors associated with risk of developing AIDS on cART.

Variable	Person follow-up time (yrs)	Number of events	Crude event rate (/1000 py), 95% CI	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Sex							
Male	3467	193	55.67 (48.09 - 64.10)	1.06(0.89-1.25)	0.53	-	-
Female	7770	406	52.25 (47.29 - 57.59)	1.00			
Age							
≥ 40 years	5161	268	51.93 (45.90 - 58.53)	1.00 (0.94-1.07)	0.90	-	-
<40 years	6082	331	54.42 (48.72 - 60.61)	1.00			
WHO stage							
3 or 4	7803	477	61.13 (55.77 –66.87)	1.73(1.37-2.18)	<0.0001	1.45(1.13-1.86)	0.0031
1 or 2	2370	84	35.44 (28.27 -43.88)	1.00		1.00	
Baseline CD4 strata							
<200	7706	481	62.42 (56.96 -68.25)	2.00(1.64-2.45)	<0.0001	1.87(1.49-2.35)	<0.0001
>200	3462	117	33.80 (27.95 -40.50)	1.00		1.00	
Baseline BMI							
<16 kg/m^2	901	99	109.9 (89.3 -133.8)	1.91(1.54-2.38)	<0.0001	1.53(1.20-1.94)	0.0005
≥16 kg/m^2	9189	483	52.56 (47.98 - 57.47)	1.00		1.00	
Baseline Hb							
<8g/dl	1176	87	73.98 (59.25 - 91.25)	0.92 (0.83-1.01)	0.06	1.01(0.78-1.31)	0.94
≥8g/dl	8590	494	57.51 (52.55 - 62.81)	1.00		1.00	
NRTI backbone							
D4T plus 3TC	5276	322	61.03 (54.55 –68.07)	1.18(1.01-1.39)	0.04	1.08 (0.90-1.30)	0.09
AZT plus 3TC	5940	274	46.13 (40.83 -51.93)	1.00		1.00	
NNRTI							
Efavirenz	6120	330	53.92 (48.26-60.06)	0.92(0.78-1.08)	0.29	-	-
Nevirapine	5013	261	52.06 (45.94 -58.78)	1.00			
Adherence							
Poor	3835	237	61.80 (54.18 -70.18)	1.17(0.99-1.38)	0.06	1.06(0.89-1.27)	0.51
Excellent	6345	363	57.21 (51.48 –63.41)	1.00		1.00	

Factors associated with loss to follow up

Nine hundred and fifty-eight (958) patients were lost to follow up (24.0%). Of the 967 patients identified as lost, 131 (13.7%) never returned for further visits after the first clinic visit when ART was dispensed. The Kaplan Meier estimated cumulative incidence of loss-to-follow up at 6, 12, 36 and 72 months were 12.7%, 15.5%, 22.6% and 29.6% respectively.

Factors associated with loss to follow up are shown in Table 4.7. Risk factors significantly associated with loss to follow up in univariate analysis were male gender, advanced clinical disease, starting CD4 count lower than 200, severe anaemia with Hb<8.0g/dl at baseline, low BMI of less than 16kg/m^2, initiating backbone NRTI of D4T plus 3TC backbone, use of efavirenz (HR of 1.20, 95%CI: 1.05 – 1.36). Patient age and residence outside Kumasi (where the HIV clinic is situated) were not significantly associated with risk of loss to follow up. In the adjusted Cox regression analysis, risk of loss to follow up remained significantly associated with male gender, WHO clinical stages III or IV, CD4 T-cell count at time of ART initiation below 200 cells/mm^3, BMI below 16kg/m^2 and initiating an NRTI backbone comprising of D4T plus 3TC.

Table 4.7. Univariate and multivariate factorial analysis of factors associated with loss to follow-up on cART.

Variable	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Sex				
Male	1.36 (1.19 – 1.55)	<0.0001	1.39 (1.18 – 1.62)	0.0001
Female	1.00		1.00	
Age				
≥ 40 years	0.98 (0.86 – 1.11)	0.76	-	-
<40 years	1.00			
WHO stage				
3 or 4	1.72 (1.43 – 2.06)	<0.0001	1.40 (1.14 – 1.70)	0.001
1 or 2	1.00		1.00	
Baseline CD4 strata				
<200	1.67 (1.44 – 1.95)	<0.0001	1.41 (1.18 – 1.68)	0.0001
>200	1.00		1.00	
Baseline BMI				
<16 kg/m^2	1.96 (1.64 – 2.33)	<0.0001	1.62 (1.33 – 1.96)	<0.0001
≥16 kg/m^2	1.00		1.00	
Baseline Hb				
<8g/dl	1.56 (1.32 – 1.85)	<0.0001	1.19 (0.98 – 1.45)	0.10
≥8g/dl	1.00		1.00	
NRTI backbone				
D4T plus 3TC	1.34 (1.18 – 1.52)	<0.0001	1.17 (1.01 – 1.36)	0.04
AZT plus 3TC	1.00		1.00	
NNRTI				
Efavirenz	1.20 (1.05 – 1.36)	0.006	1.05 (0.89 – 1.22)	0.57
Nevirapine	1.00		1.00	
Residence location				
Outside Kumasi	1.12 (0.97 – 1.28)	0.12	-	-
Within Kumasi	1.00			

The incidence, causes and risk factors associated with mortality.

There were 324 deaths over the 11,263.8 person follow-up years giving a crude (95% CI) event rate of 28.83 (25.78 – 32.15) deaths per 1000-person follow up years. The median (range) time to death was 2 months (0 – 66) by Mann Whitney's U-test. 202 (62.3%) of deaths occurred within the first 90-days of initiation of therapy, 88 (27.2%) from month 4 to month 12, 16 (4.9%) within the second year, 9 (2.8%) within the third year, 3 (0.9%) within the fourth year and 6 (1.9%) within the fifth year of follow up. The Kaplan Meier estimated cumulative incidence of mortality at 6, 12, 36 and 72 months were 6.8%, 7.9%, 9.0% and 9.8% respectively.

On univariate analysis, factors significantly associated with hazard of death included male gender, advanced clinical stage of HIV disease (Stage 3 or 4), baseline CD4 strata <200 cells/mm^3, baseline BMI of below 16kg/m^2, baseline haemoglobin of less than 8g/dl, starting D4T plus 3TC, starting EFV-based ART and poor adherence to therapy. As shown in Table 4.8, significant factors associated with mortality in this model were male gender with an adjusted HR (95% CI) of 1.69 (1.29-2.21), advanced HIV disease-stages 3 or 4 with an adjusted HR (95% CI) of 2.20 (1.42 – 3.41), low CD4 strata below 200 cells/mm^3 with an adjusted HR (95% CI) of 2.39 (1.64-3.49), baseline BMI below 16kg/m^2 with an adjusted HR (95% CI) of 2.60 (1.92-3.53) and starting on an NRTI backbone of D4T plus 3TC with an adjusted HR of 1.60 (1.21-2.11). Starting on an EFV-based ART compared with NVP-based ART in this model was not found to be significantly associated with risk of death with an adjusted HR of 1.15 (0.87 – 1.51), p=0.32. Other baseline factors associated with significant risk of mortality identified in univariate analyses included low estimated glomerular filtration rate (elaborated further in next sub-section), low total serum cholesterol with an HR of 3.47 (2.95 – 12.58), p<0.0001 (not shown) and raised liver transminases – ALT >40IU/l and AST >40IU/l with unadjusted HRs of 1.75 (95% CI of 1.42 to 2.49), p<0.0001 and 1.66 (95%CI of 1.29 to 2.14), p<0.0001 respectively.

In a series of 188 deaths, the causes of death were identified from patient records and the frequencies of the conditions certified by physicians to be the cause of mortality are as shown in Table 4.9. The leading causes of death in this subset of patients were pulmonary tuberculosis, severe diarrhoea with hypovolemic shock, severe anaemia, pneumonia and sepsis.

Table 4.8. Univariate and multivariate analysis of factors associated with risk of death on cART among Ghanaians.

Variable	Patient follow-up time (years)	Number of events	Crude event rate (/1000 person years)	Unadjusted hazard ratio (95% CI)	p-value	Adjusted hazard ratio (95% CI)	p-value
Sex							
Male	3467	188	54.23 (46.75 - 62.56)	1.71(1.37-2.14)	<0.0001	1.69(1.29-2.21)	0.0001
Female	7770	136	17.50 (14.69 - 20.70)	1.00		1.00	
Age							
≥ 40 years	5161	143	27.71 (23.35 – 32.64)	0.94(0.75-1.17)	0.55	-	-
<40 years	6082	180	29.60 (25.43 – 34.25)	1.00			
WHO stage							
3 or 4	7803	277	35.50 (31.44 – 39.94)	3.52 (2.34-5.30)	<0.0001	2.20(1.42-3.41)	0.0004
1 or 2	2370	25	10.55 (6.82 -15.57)	1.00			
Baseline CD4 strata							
<200	7706	276	35.81 (31.72 – 40.30)	3.13 (2.29-4.29)	<0.0001	2.39 (1.64-3.49)	<0.0001
>200	3462	45	13.00 (9.48 – 17.39)	1.00			
Baseline BMI							
<16 kg/m^2	901	88	97.67 (78.33 - 120.33)	3.79 (2.95-4.85)	<0.0001	2.60 (1.92-3.53)	<0.0001
≥16 kg/m^2	9189	214	23.29 (20.27 – 26.63)	1.00			
Baseline Hb							
<8g/dl	1176	76	64.63 (50.92 – 80.89)	2.43(1.88-3.16)	<0.0001	1.28 (0.95-1.75)	0.11
≥8g/dl	8590	227	26.43 (23.10 – 30.10)	1.00			
NRTI backbone							
D4T plus 3TC	5276	216	40.94 (35.66 – 46.78)	2.04 (1.62-2.58)	<0.0001	1.60 (1.21-2.11)	0.001
AZT plus 3TC	5940	106	17.85 (14.61 – 21.58)	1.00			
NNRTI							
Efavirenz	6120	212	34.64 (30.13 – 39.63)	1.43(1.14-1.81)	0.0024	1.15 (0.87-1.51)	0.32
Nevirapine	5013	108	21.54 (17.67 – 26.01)	1.00			
Adherence							
Poor	3835	147	38.33 (32.39 – 45.05)	1.52 (1.22-1.89)	0.0002	1.21 (0.95-1.56)	0.12
Excellent	6345	177	27.90 (23.94 – 32.32)	1.00			

Table 4.9 Causes of death among 188 patients who died on first line cART in descending order of frequency.

Cause of death	Frequency
Pulmonary tuberculosis	37
Diarrhoea with hypovolaemic shock	30
Severe anaemia	19
Pneumonia	18
Sepsis	16
Cerebral toxoplasmosis	12
HIV Encephalopathy	7
TB Immune reconstitution inflammatory syndrome	7
Disseminated Kaposi sarcoma	6
Enteric fever	4
End stage kidney failure	4
Lactic acidosis	4
Bacterial meningitis	4
Chronic liver disease	2
Cryptococcal meningitis	2
Steven's Jonhnsons syndrome due to nevirapine therapy	2
Miscellaneous*	14
TOTAL	188

***** Miscellaneous comprises of 1 case each of acute abdomen, amoebic liver abscess, CNS lymphoma, gluteal abscess, hepatocellular carcinoma, HBV flare, high grade Non-Hodgkin's lymphoma, hyperglycemic hyperosmolar syndrome, strangulated umbilical hernia, otitis media, *Pneumocystis jirovercii* pneumonia, systemic candidiasis, tuberculous colitis, fulminant vasculitis with gangrene of toes and fingers.

The impact of baseline renal impairment on survival after initiating cART

In chapter 3, the prevalence of renal impairment determined by estimated glomerular filtration rate of <60ml/min using the Cockroft-Gault and CKD-EPI formulae were 38.8% and 13.9% respectively. However during cART, very infrequent serum creatinine measurements were performed thus precluding analysis of trends in eGFR over time. For instance, out of 3,136 measurements at baseline only 432 had repeat creatinine measurements at 2 months, 220 at 6 months and 93 at 12 months. A limited analysis of changes in eGFR over the first 8 weeks was performed with a trend towards improvement in eGFR among patients who started with mild to moderate renal impairment (data not shown).

However, in survival analysis using baseline eGFR, there was a significant trend towards increased risk of death according to decreasing baseline eGFR as shown in Figures 4.3 (missing = censored analysis) and 4.4 (missing = death analysis). In a multivariate Cox proportional hazard analysis (missing = censored analysis) with adjustment for gender, age, baseline CD4 counts, WHO clinical stage, NNRTI backbone and NNRTI, there was a significantly increased hazard of death with an eGFR of <60ml/min using either the Cockroft-Gault or the CKD-EPI formulae with adjusted HRs of 1.46 (95%CI of 1.31 to 1.63), $p<0.0001$ and 1.29 (95%CI of 1.16-1.44), $p<0.0001$ respectively for each tertile lower than an eGFR of 90ml/min. Tables 4.10A and 4.10B summarise the impact of baseline eGFR on the risk of death on cART using either the Cockroft-Gault or the CKD-EPI formulae respectively, with similar trends also observed using the MDRD formula (not shown).

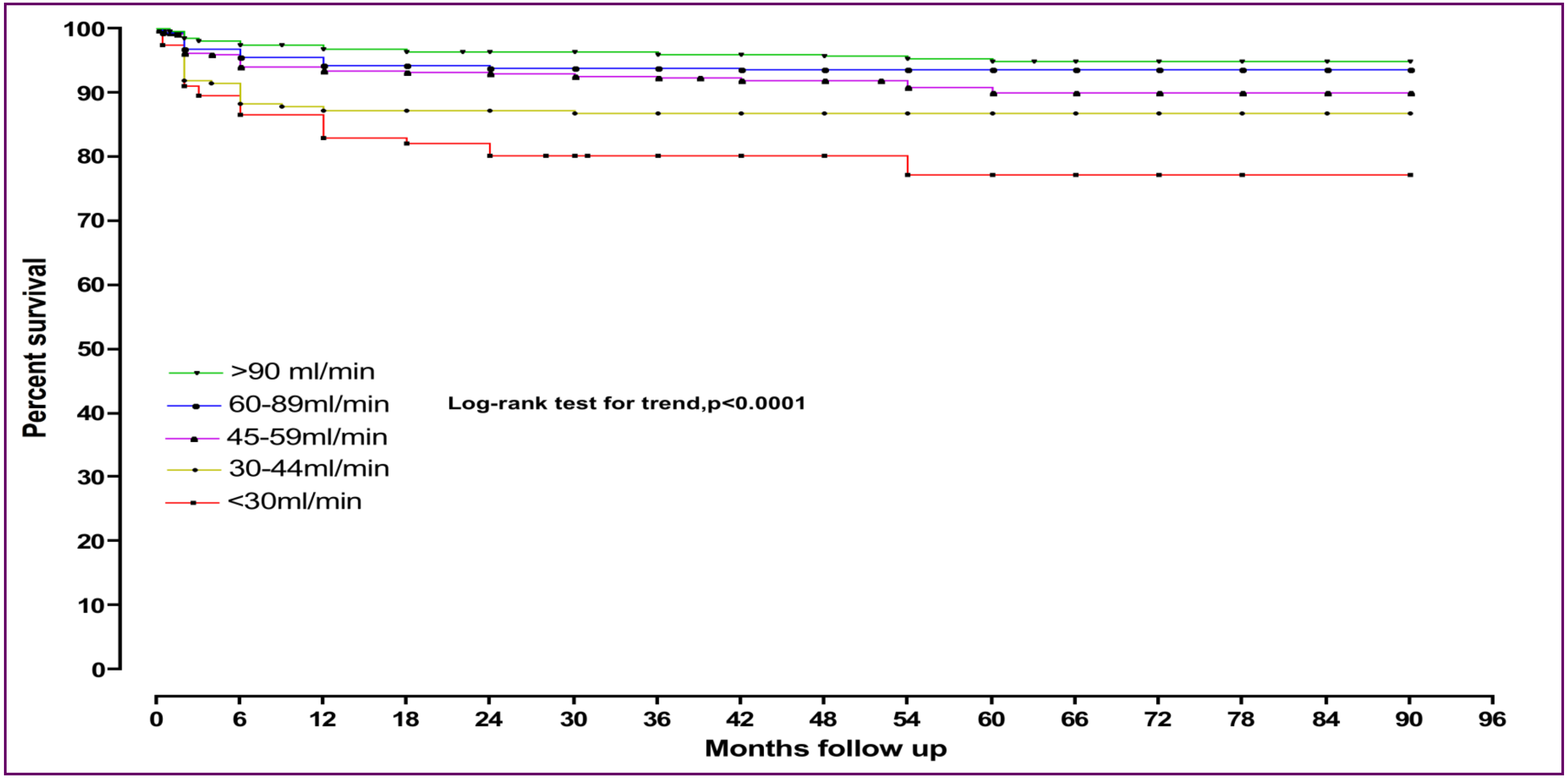


Figure 4.3. Kaplan –Meier risk analysis for death according to baseline estimated glomerular filtration rate using the Cockroft-Gault formula (missing patient were censored).

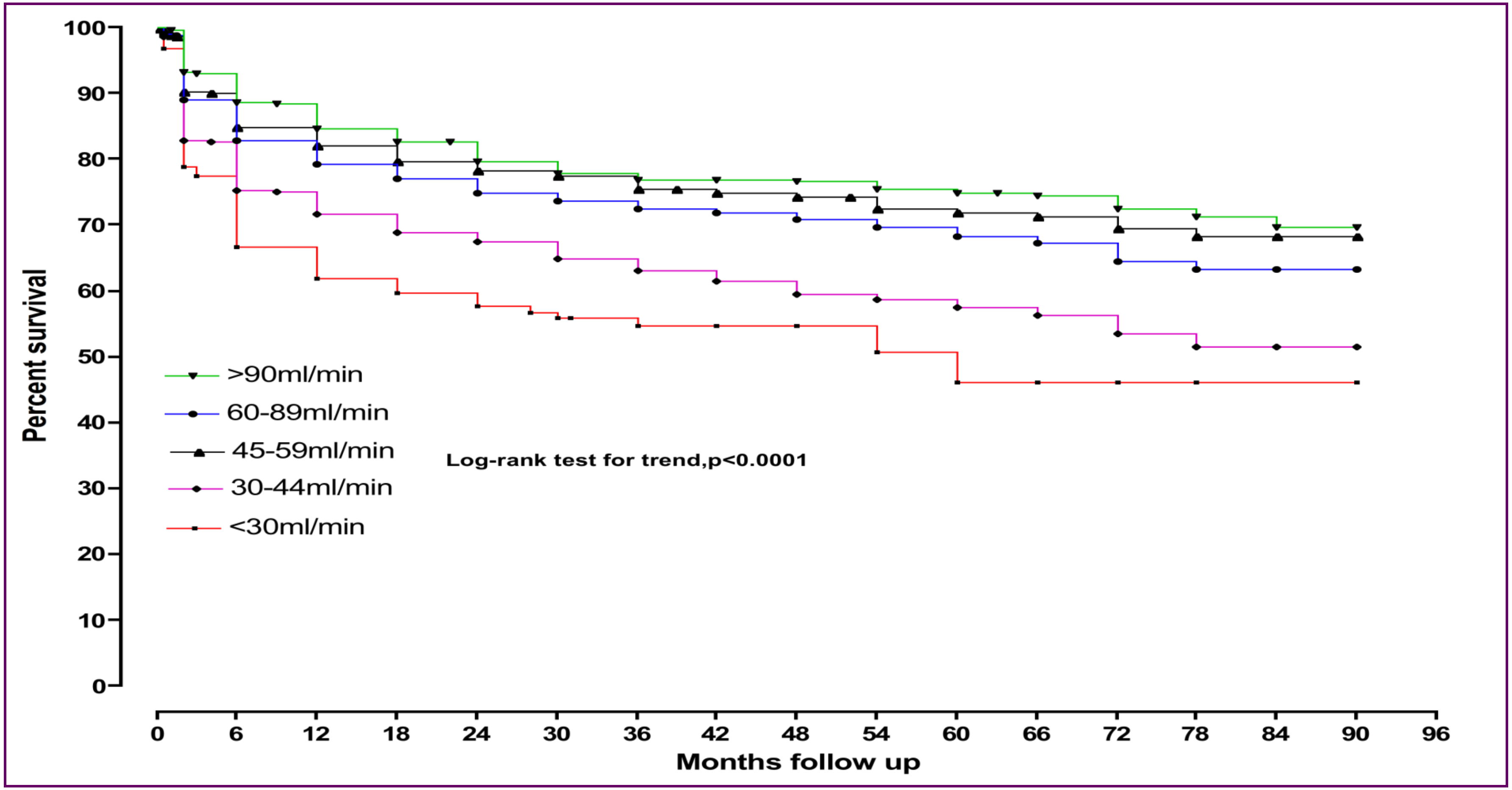


Figure 4.4. Kaplan –Meier risk analysis for death according to baseline estimated glomerular filtration rate using the Cockroft-Gault formula (missing patient = death).

Table 4.10A. Cox proportional hazard regression analysis for risk of death on cART according to estimated glomerular filtration rate (by Cockroft-Gault equation) at baseline.

	Missing = Censored (Primary analysis)				Missing = death (Sensitivity analysis)			
Variable eGFR	**Unadjusted HR**	**p-value**	**Adjusted HR**	**p-value**	**Unadjusted HR**	**p-value**	**Adjusted HR**	**p-value**
>90ml/min	1.00		1.00		1.00		1.00	
60-89ml/min	1.34 (0.86-2.10)	0.19	1.29 (0.83-2.03)	0.26	1.22 (1.01-1.47)	0.04	1.20 (1.00-1.45)	0.05
45-59 ml/min	2.14 (1.35-3.38)	0.0012	1.95 (1.21-3.12)	0.0057	1.16 (0.94-1.44)	0.18	1.09 (0.87-1.36)	0.46
30-44ml/min	3.41 (2.13-5.44)	0.0000	2.55 (1.55-4.17)	0.0002	1.89 (1.51-2.36)	0.0000	1.64 (1.29-2.07)	0.0000
<30ml/min	5.75 (3.45-9.59)	0.0000	4.08 (2.34-7.11)	0.0000	2.47 (1.88-3.24)	0.0000	2.05 (1.52-2.75)	0.0000

Table 4.10B. Cox proportional hazard regression analysis for risk of death on cART according to estimated glomerular filtration rate (by CKD-EPI equation) at baseline.

	Missing = Censored (Primary analysis)				Missing = failure (Sensitivity analysis)			
Variable eGFR	**Unadjusted HR**	**p-value**	**Adjusted HR**	**p-value**	**Unadjusted HR**	**p-value**	**Adjusted HR**	**p-value**
>90ml/min	1.00		1.00		1.00		1.00	
60-89ml/min	1.13 (0.84-1.52)	0.43	1.29 (0.96-1.75)	0.09	0.99 (0.85-1.14)	0.85	1.08 (0.93-1.26)	0.30
45-59 ml/min	1.77 (1.14-2.76)	0.01	1.84 (1.18-2.89)	0.0076	1.36 (1.07-1.73)	0.01	1.41 (1.11-1.79)	0.0056
30-44ml/min	2.30 (1.32-4.01)	0.0034	1.99 (1.13-3.50)	0.018	1.69 (1.24-2.30)	0.0009	1.57 (1.15-2.16)	0.0046
<30ml/min	3.24 (1.94-5.41)	0.0000	2.82 (1.68-4.74)	0.0001	1.98 (1.45-2.70)	0.0000	1.78 (1.30-2.43)	0.0003

Adjusted analysis includes adjustment for gender, age, baseline CD4 counts, WHO clinical stage, NRTI backbone and NNRTI base

Adherence to cART: Adherence was assessed using pill counts at every clinic visit and classified as adherent if patient had taken >95% of prescribed pills and non-adherent if patient had taken <95%. A patient was classified as non-adherent if there is any reported history of poor compliance to therapy during follow-up. 1,509 (38%) patients who had at least one visit after initiating cART had a history of poor adherence. Risk factors associated with a significant odds of poor adherence were advanced WHO clinical stage with each increase in clinical stage the OR (95% CI) was 1.15 (1.06 – 1.26), p=0.002; starting therapy with a BMI lower than 16kg/m^2 is associated with OR (95% CI) of 1.33 (1.10 – 1.61), p=0.0041, for each 100 cells increase in baseline CD4 count the OR (95% CI) of poor adherence was 0.86 (0.81 – 0.91), p<0.0001 and initiating an efavirenz-based ART is associated with an OR (95% CI) of 1.14 (1.00 – 1.30), p=0.05. Gender, age and NRTI backbone used was not associated with significant odds of poor adherence. In a multiple logistic regression model the only factor significantly associated with poor adherence was baseline CD4 count where for every 100 cells increase in CD4 count, there is a 13% decrease in risk for poor adherence with an OR (95% CI) of 0.87 (0.82 – 0.93), p=0.0001.

Rates of major clinical events on cART: In summary Figure 4.5 graphically depicts the overall decline in the period prevalence rates of AIDS-defining events, non-AIDS clinical events, mortality and loss to follow up over time on cART. The highest incidence rates of these events were observed within the first two months of initiating cART with rates of 22.1 non-AIDS clinical events per 100 patients, 10.7 lost-to-follow up per 100 patients, 5.9 AIDS defining events per 100 patients and 5.0 deaths per 100 patients under follow-up. Overall, the Kaplan-Meier estimates of percentage attrition from treatment programme due to either death or loss to follow up at 6, 12, 24, 48, 72 and 90 months were 17.3%, 20.7%, 25.1%, 29.0%, 34.5% and 36.5% respectively.

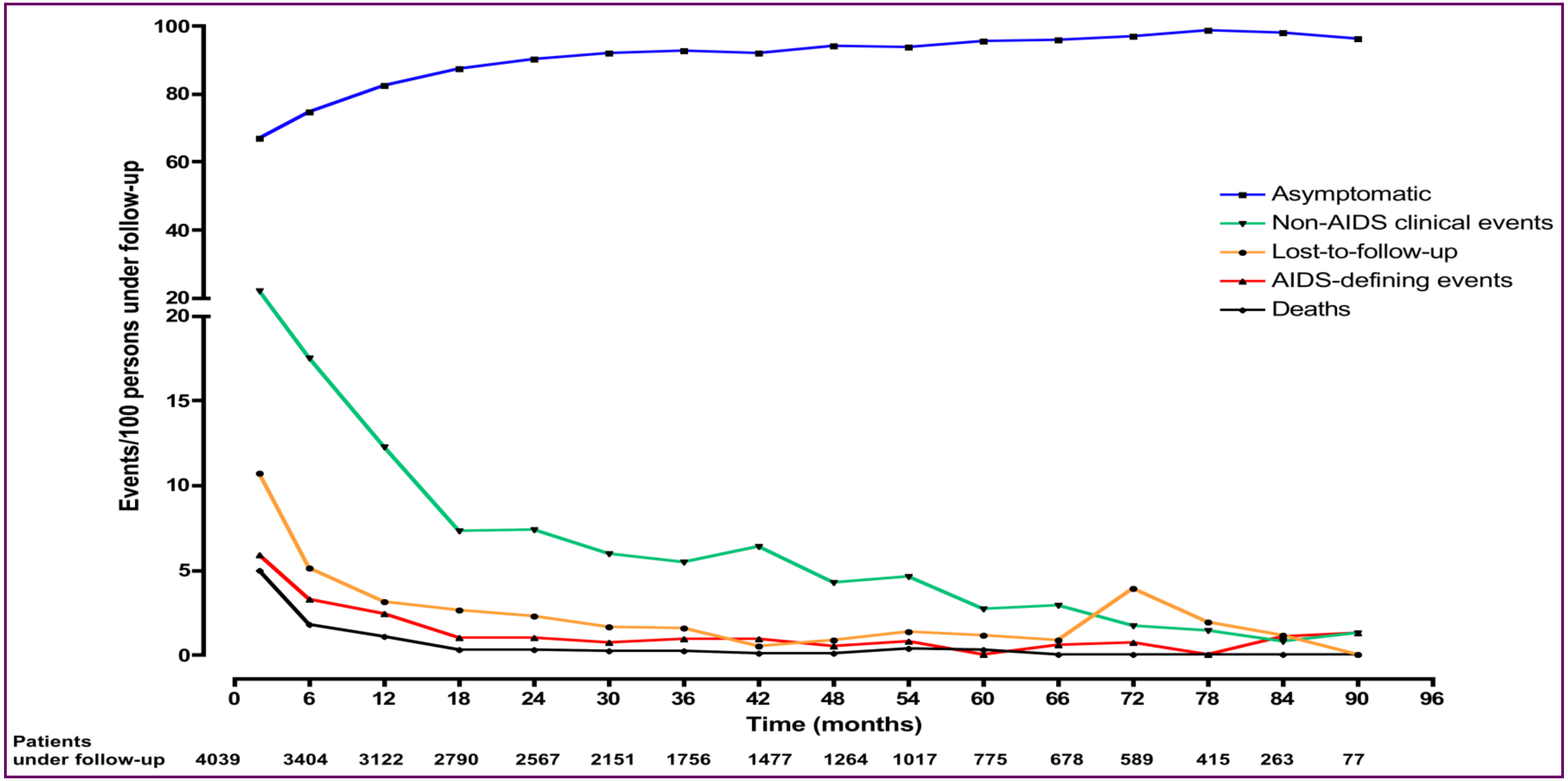


Figure 4.5. Six monthly incidence rates of Non-AIDS and AIDS events, deaths, loss to follow up and asymptomatic events among Ghanaian HIV-infected patients on long-term cART. Non-AIDS events comprised all medical conditions which are non-AIDS defining by WHO criteria.

4.4 DISCUSSION

The data presented in this chapter shows that there is a sustained and robust immunological recovery on combination antiretroviral therapy among this Ghanaian cohort of HIV Infected patients. This immune restoration occurs in the accompaniment of improvement in morbidity as evidenced by the increasing proportion of patients who became asymptomatic over the course of treatment, the increasing body mass indices of patients, and the steady decline in the incidence of deaths, patients lost to follow-up, AIDS-defining and non-AIDS defining clinical events. Attrition from the programme due mainly to deaths and loss-to-follow up as well as AIDS-defining events occurred predominantly within the first year of treatment and were predicted by clinical and laboratory indicators of advanced HIV disease at initiation of cART. The data presented also shows a strong and independent, dose-response association between baseline renal impairment assessed by eGFR from creatinine measurement and the subsequent risk of death after commencing cART among this cohort.

Several studies from both developed[439-443] and developing countries[426, 444-449] have reported short-, medium- and recently long-term CD4 responses on cART. The findings from this study shows that within a programme setting in Sub-Saharan Africa, immunological responses to cART is sustainable over the long-term on a predominantly limited repertoire of first-line therapy. As expected, the median (IQR) CD4 counts at initiation of therapy of 133 (50 – 218) cells/mm^3 was low and comparable with those from several cohorts from developing countries[444-454] and serves as an indicator of the advanced stages of immunosuppression at which cART is initiated in these settings[455]. This notwithstanding, cART was associated with a near doubling in median CD4 counts within 6 months of therapy followed by a more gradual and sustained increases throughout the period of observation for patients remaining under care. The initial profound recovery in CD4 counts on cART is thought to be due to the redistribution of cells from lymphoid tissue over the first few months of therapy as suppression of viral replication reduces immune activation[456-459]. The vigorous restitution of CD4 cell counts within the first six months of therapy was as expected accompanied by the occurrence of the immune reconstitution inflammatory syndrome in some patients of which tuberculosis was the commonest reported cause followed by herpes zoster and cerebral toxoplasmosis. Admittedly, compared with other cohorts where the reported frequency of IRIS has varied between 10% to 25% of all patients initiating cART[360, 460-464], the overall frequency of 1.1% of IRIS reported among this cohort is low, and probably a reflection the well-known difficulties in the diagnosis of this clinical syndrome within settings of limited laboratory support. Events considered as IRIS were those clinicians felt they had sufficient clinical and laboratory evidence to corroborate their suspicions.

Since the phenomenon of IRIS represents an exaggerated immune response to tissue antigens of specific pathogens during immune recovery on cART, it is uncertain whether it could be classified as a failure of cART. However for patients who stayed on cART, 407 (10.1%) developed immunological failure according to WHO guidelines. It is obvious that the spectra of clinical events classified as IRIS (Table 4.1) overlaps very well with those diagnosed as AIDS-defining events (Table 4.5) of which 681 were recorded among the entire cohort at a frequency of 17%. AIDS-defining events on cART occurred at a median time of 6 months (range 2 to 90 months), were predicted by low CD4 cell counts, prior AIDS and low BMI at initiation of therapy and were associated with increased risk of subsequent attrition from the programme in 47.2% of events. These suggest that the occurrence of AIDS-defining events in the proximal phases of cART is an indicator of early treatment failure and is representative of an inexorable progression of HIV disease for which cART may be incapable of halting, even when the appropriate treatment for the opportunistic infection is concurrently commenced. The implication is that cART should be started much earlier, when patients are diagnosed earlier as has been suggested by others[455, 465].

The crude incidence rates of Non-AIDS-defining events of 3.64/1000 person-years (95% CI of 2.61 – 4.95/1000 person years) is lower compared to that of cohorts from Botswana[438] and the United States[438] of 10.0 (95% CI of 6.3-15.9/1000 py, n=650) and 12.4 (95% CI of 8.4 to 18.4 py, n=1,129) respectively, that of a multicentre Latin American cohort[466] of 8.4/1000 person-years and that of the Eurosida cohort[467] of 17.7 (95% CI of 16.6 to 18.7/1000 py, n=12,844). Of the 41 NADEs in this Ghanaian cohort, hepatic disorders (n=20) were the commonest NADE followed by cardiovascular events (n=10), end-stage renal disease (n=6) and non-AIDS malignancies of which hepatocellular carcinoma (n=4) and one case of oesophageal carcinoma were reported. The order of events were different from those in the cohort from Botswana where out of 18 NADEs, 9 were cardiovascular, 4 were renal, five were malignancies and none were of hepatic in etiology[438]. As a composite outcome measure, non-AIDS events are a heterogeneous collection of several end-points with multifactorial aetiologies and risk factors such as increasing age on cART, presence of co-morbid medical disorders such as hypertension, diabetes mellitus and dyslipidaemia as well as lifestyle, for example smoking or alcohol abuse, and also the presence of chronic hepatitis B co-infection and chronic vascular inflammatory state due to persistence of HIV viral replication[468-477]. The multi-dimensional composition of risk factors for NADEs together with the relative limited observation of events and some missing data at baseline and follow up restricted analysis of risk factors for these events in this cohort.

Malaria was the leading cause of non-AIDS-defining infectious morbidity on cART and was often treated with artemisinin-based antimalarial therapy leading to resolution of symptoms. However the safety and effectiveness of this class of antimalarials among HIV-infected patients on cART has not been assessed within the Ghanaian context. Thus the impact of pharmacodynamic and pharmacokinetic interactions between artemisinins and efavirenz is explored further in chapter 7. The fact that within this HIV cohort, tuberculosis and malaria were the leading causes of AIDS and non-AIDS infectious morbidity respectively underpins these three infectious diseases as the most important public health challenges Sub-Saharan Africa is attempting to overcome to enhance longevity of its population.

Unlike in the developed countries where AIDS-related mortality on cART is being superseded by Non-AIDS-related mortality[477], causes of death in this cohort were predominantly driven by AIDS-related events of which tuberculosis was the leading cause followed by diarrhoea, severe anaemia and pneumonia as in other cohorts from developing countries. Of the NADEs, end-stage kidney disease and liver-related disease were among the common causes of mortality. Renal impairment, defined by eGFR of <60ml/min, was very frequent prior to initiation of therapy with a prevalence of 38.8% and 13.9% respectively using either the Cockroft-Gault or the CKD-EPI formulae respectively. This study shows a graded increase in the risk of death according to baseline eGFR with adjusted HRs of 4.08 (95% CI 2.34-7.11), 2.55 (1.55-4.17) and 1.95 (1.21-3.12) at eGFRs of <30ml/min, 30-44ml/min and 45-59ml/min compared with

>90ml/min (Table 4.10A). This trend was consistently demonstrated whether eGFR was estimated with Cockroft-Gault (Table 4.10A) or CKD-EPI (Table 4.10B) and whether missing patients were censored (Figure 4.4) or were considered as dead (Figure 4.5). These findings are in agreement with the recently published data from the UK CHIC cohort[478]. It is also noteworthy that 6 deaths were attributed directly to ART related toxicity: 2 cases of Stevens Johnson's syndrome from nevirapine and four cases of clinically suspected lactic acidosis from stavudine. ART toxicity is a cardinal factor influencing the durability and therefore the effectiveness of cART and this is explored in some detail in the next chapter. Use of a stavudine backbone was independently associated with increased risk of death (Table 4.8) and loss-to-follow up (Table 4.7), hence the withdrawal of this antiretroviral from the Ghanaian ART programme is justified and its associated risk of mitochondrial-related toxicity with long-term use is explored further in chapter 5. Given the high prevalence of renal impairment among this cohort, replacement of stavudine by tenofovir backbone should however be approached with caution due to well known association between tenofovir and risk for renal tubular toxicity. It is encouraging that the DART study has provided some reassuring data on the safety of tenofovir among Africans[395], nevertheless regional differences in prevalence of renal impairment across Africa should be borne in mind. More frequent assessment of renal function using urine dipsticks and creatinine measurements are needed to detect worsening of renal function should this occur.

The analyses presented in this chapter have limitations worth noting. The trajectories of CD4 and body mass index over time reflect those of patients who remained on cART and thus are influenced by survivor bias. Baseline predictors of the trajectories of CD4 counts were not explored in this chapter but are considered in chapter 6 where the effectiveness of efavirenz-based cART is compared with that of NVP-based cART. Also the outcomes of renal function after initiation of cART could not be assessed because of less frequent monitoring of renal function by creatinine measurements. Indeed in a very limited analysis of patients who had repeat measurements of creatinine performed within 8 weeks of starting cART, there was a trend towards a down-staging of baseline renal impairment among those with eGFR of <60ml/min as has been previously shown[478]. Also of note is the fact that adherence to therapy was assessed from records of pill counts and graded into a binary outcome of excellent or poor. Previous studies have modelled risk factors for adherence using either the GEE[479] or log-binomial models[446] but there was failure to achieve convergence using these methods for reasons still not apparent to the author. Corroboration with pharmacy refills could have improved the assessment of adherence. Furthermore the sources of ascertainment of causes of deaths were mostly done by verbal autopsy by relatives and may not have been accurate, others were inferred from the last diagnosis of the patients before death occurred as well as those from medical certificates of death. In spite of these the specific causes of death could be verified in only 188 (58%) out of 324 events. Similarly, the classification of AIDS-defining events, IRIS and NADEs were influenced by availability of data and arrived at by consensus between the author and his local supervisor (ROP) since no structured- proforma has been designed to capture and classify these events in the ART programme.

In summary, cART is effective over the long-term among this Ghanaian cohort of HIV-infected patients. Two deaths were due to nevirapine related toxicity but the risk of immunological failure, death, loss-to-follow up and disease progression were similar between nevirapine and efavirenz. The effectiveness of these two NNRTIs are directly compared in chapter 6 of this dissertation. Stavudine was associated with increased risk of death and loss-to-follow up supporting the recommendations to withdraw it from ART programmes across Africa. Within the constraints of limited resources as it pertains to the ART programme in Ghana, cART was associated with sustained and durable immunological recovery, an improvement in the morbidity from HIV-infection and trend towards reduction in mortality over the long-term among patients remaining on therapy and under care.

CHAPTER FIVE

Incidence and risk factors associated with toxicity on first line anti-retroviral therapy among Ghanaian HIV infected patients.

Introduction

Over the last several years, a successful scaling-up of antiretroviral therapy in most resource-constrained countries has occurred with currently over 5 million individuals on treatment[1]. The availability of cheap, generic fixed-dose combination (FDC) has been a key issue in achieving this[480]. In line with WHO recommendations at the start of the ART roll-out, almost all national programmes have implemented first line treatment consisting of a FDC containing stavudine (D4T)/ zidovudine (AZT), lamivudine (3TC) and nevirapine (NVP)/ efavirenz (EFV)[481]. The continual use of these first line cART regimens - a measure of their durability - is dependent on their efficacy and toxicity profile over long-term usage. Adverse effects have been reported with all antiretrovirals and are one of the most common reasons for discontinuation of treatment[149, 482, 483]. Some events such as hypersensitivity, cutaneous and hepatotoxic reactions to NNRTI occur rapidly, within the first few months of starting treatment. Others such as lipoatrophy on thymidine NRTI evolve over long-term usage, still others such as anaemia due to zidovudine may occur unpredictably.

Surveillance data on long-term toxicity of antiretrovirals are important within the programmatic settings where cART is administered to inform policy change where necessary. For instance, the increasing reports of stavudine-related mitochondrial toxicities, including neuropathy, lactic acidosis and lipoatrophy led to the WHO in its 2006 guidelines to recommend phasing-out D4T and replacing it with alternative drugs[484]. It is common practise, based on treatment recommendations, to substitute drugs within a particular class on account of toxicity, but risk of recurrent adverse events due to cross-reactivity among drug classes particularly the NNRTIs has not been explored within cohorts in Africa. Given that toxicity on ART may cause patients to adhere less to therapy and therefore engender treatment failure it is important to explore risk factors associated with the incidence of severe toxicity among the currently available first line therapy. The aims of this chapter are first, to evaluate the incidence rates and risk factors for antiretroviral therapy related-toxicities; second, to compare the rates of discontinuation within two ART classes due to toxicity (i.e efavirenz vs nevirapine and stavudine vs zidovudine); third, to examine the impact of specific ART-related toxicity on adherence to therapy. Since the focus of this dissertation is to examine the effectiveness of efavirenz with nevirapine as a comparator, the analysis for each specific toxicity in this chapter is performed with these two medications in consideration regardless of whether they are implicated as the cause of the toxicity or not.

Methods

Please refer to chapter 2 sections 2.5.

Results

Incidence and timing of Specific ART-related toxicity

Eight hundred and ninety-five (895) patients experienced a total of 1,627 ART related toxicities over 11,236.8 person years of follow up. As shown in Table 5.1, anaemia, skin rash, neuro-psychiatric disorders, peripheral neuropathy, hepatotoxicity and lipoatrophy were the commonest ART associated toxicities recorded. An analysis of the incidence rates and risk factors associated with these six frequently reported ART toxicities are found below:

Table 5.1: Frequencies, incidence rates and median time of first occurrence of specific toxicities on ART among Ghanaian HIV patients.

Toxicity	Number of events n= 1,627	Frequency of events	Median (range) time in months	Incidence rate /100 person years follow up (95% CI)
Severe anaemia	675	41.5%	2 (2 – 78)	6.01 (5.56 – 6.48)
Skin rash	299	18.4%	2 (2 – 48)	2.66 (2.37 – 2.98)
Neuropsychiatric disorders	235	14.4%	2 (2 – 78)	2.09 (1.83 –2.38)
Peripheral neuropathy	181	11.2%	6 (2 – 72)	1.61 (1.38 – 1.86)
Hepatotoxicity	160	9.8%	12 (2 – 84)	1.42 (1.21 – 1.66)
Lipoatrophy	40	2.5%	42 (2 – 66)	0.36 (0.25 – 0.48)
Ptylism	14	0.9%		0.12 (0.07 – 0.21)
Gastrointestinal disorders	12	0.7%		0.11 (0.06 – 0.19)
Lactic acidosis	4	0.2%		0.04 (0.01 – 0.09)
Hyperpigmentation	3	0.2%		0.03 (0.01 – 0.08)
Myalgia	3	0.2%		0.03 (0.01 – 0.08)
Pancreatitis	1	0.1%		0.01 (0.00 – 0.05)

(1) Incidence and risk factors for developing severe anaemia on ART among Ghanaian HIV infected patients

Incidence rates: 527 patients experienced a total of 675 episodes of severe anaemia defined by haemoglobin concentration of <8.0g/dl, giving an event rate (95% CI) of 6.01 (5.56 - 6.48 per 100 person -years). 427 patients experienced 1 episode of severe anaemia while 78 had 2 episodes, 9 had 3 episodes, 5 had 4 episodes, 4 had 5 episodes, 3 had 6 episodes and 1 patient had 7 episodes of severe anaemia during follow-up. The median time to onset of first episode of severe anaemia was 2 months (range of 2-78 months). The median (IQR) haemoglobin concentration of patients initiating zidovudine-containing ART was 11.0g/dl (9.9 – 12.3) compared with 9.4g/dl (8.2 – 10.9), p<0.0001 for those initiating stavudine-containing regimen. Overall, the median time to first episode of severe anaemia was 2 months (range of 2 to 78 months), that for zidovudine-recipients of 2 months (range, 2-78 months) was not significantly different from D4T recipients of 2 months (range, 2-72 months), p=0.88.

To identify the risk factors for this common toxicity, a general model for predicting the occurrence of severe anaemia defined as haemoglobin concentration below 8g/dl is first presented. This is followed by two separate models the explores the risk factors for developing severe anaemia among two categories of patients: those whose haemoglobin concentrations were within normal limits at the start of therapy and then those who had some degree of anaemia at baseline but experienced a further deterioration or persistence of anaemia after starting ART.

(i) General model for predicting severe anaemia on cART: The risk factors identified on univariate and multivariate Cox proportional hazard analyses in association with severe anaemia during ART are shown in Table 5.2. Baseline univariate predictors of severe anaemia included BMI below 18.5kg/m^2 with HR (95% CI) of 1.69 (1.42 – 2.01), WHO clinical stages 3 or 4 disease with HR (95% CI) of 1.79 (1.38 – 2.31), CD4 count below 200 cells/mm^3 with HR (95% CI) of 1.67 (1.36 – 2.05), initiating ART with severe anaemia HR (95% CI) of 3.49 (2.90 – 4.19). Gender, age, baseline estimated GFR and NNRTI used to initiate therapy were not significantly associated with risk of anaemia while initiating AZT-containing regimen was associated with a HR (95% CI) of 0.67 (0.57 – 0.81) of developing anaemia compared with a D4T-containing regimen. In multivariate analysis (n=3,436), factors that remained significantly associated with the risk of developing severe anaemia included advanced WHO clinical stage at initiation of ART, CD4 cell count below 200 cells/mm^3 at baseline, a BMI below 18.5 kg/m^2 at baseline and severe anaemia at baseline prior to initiating therapy.

In this model neither NRTI namely zidovudine nor stavudine was independently associated with the risk for developing severe anaemia. However, given that the recommended guidelines for management stipulate the avoidance of zidovudine in patients with haemoglobin concentration below 10g/dl, it is possible that this observation could have been driven by confounding due to an indication bias. To explore this issue further, the risk factors for developing severe anaemia was explored among those who had any degree of anaemia at initiation of therapy and those who started therapy without any degree of anaemia in separate analyses.

Table 5.2. The risk factors for developing severe anaemia on cART among Ghanaian HIV-infected patients

Predictor	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Gender				
Female	1.01 (0.84 – 1.21)	0.95	-	-
Male	1.00			
Age				
≥40 years	0.95 (0.80 – 1.12)	0.52	-	-
<40 years	1.00			
BMI				
<18.5 kg/m^2	1.69 (1.42 – 2.01)	<0.0001	1.27 (1.04 – 1.55)	0.01
≥ 18.5 kg/m^2	1.00			
WHO clinical stage				
stage 3 or 4	1.79 (1.38 – 2.31)	<0.0001	1.43 (1.09 – 1.88)	0.01
stage 1 or 2	1.00		1.00	
Baseline CD4 counts				
<200 cells/mm^3	1.67 (1.36 – 2.05)	<0.0001	1.34 (1.07 – 1.68)	0.01
≥200 cells/mm^3	1.00			
Baseline HB				
<8.0g/dl	3.49 (2.91 – 4.19)	<0.0001	2.79 (2.27 – 3.43)	<0.0001
≥ 8.0g/dl	1.00		1.00	
eGFR				
<60ml/min	0.86 (0.68 – 1.08)	0.19	-	-
≥60ml/min	1.00			
NRTI				
Zidovudine	0.68 (0.57 – 0.81)	<0.0001	0.87 (0.71 – 1.07)	0.18
Stavudine	1.00		1.00	
NNRTI				
Efavirenz	1.15 (0.96 – 1.37)	0.13	-	-
Nevirapine	1.00			

(ii) Model for predicting factors associated with the risk of developing severe anaemia among patients with some degree of anaemia at the start of cART

Included are 2,943 patients who had some degree of anaemia at baseline and at least one haemoglobin concentration determination during follow-up on cART. Figure 5.1 shows the changes in haemoglobin concentration from baseline values among this subset of patients while on cART. Overall, there was an increase in the median of change in haemoglobin concentration on cART, however among this high-risk group of patients, some experienced severe to life-threatening reductions in haemoglobin concentrations defined as a drop in haemoglobin concentration of 2.5g/dl from baseline value or haemoglobin concentration of <8g/dl. By this definition 531 (18.0%) patients had at least one episode of worsening of anaemia on cART out of which 86 patients experienced recurrent anaemia defined by at least 2 episodes of severe to life-threatening anaemia (range of 2-8 episodes) on follow-up.

Significant baseline factors associated with the risk of developing severe anaemia among this subset of patients initiating cART with some degree of anaemia included CD4 count <100 cells/mm^3, AIDS diagnosis and BMI <18.5 kg/m^2 with unadjusted HRs (95% CI) of 1.18 (1.00-1.45), 1.60 (1.24-1.97) and 1.45 (1.27-1.85) respectively while male gender was marginally significant with HR of 1.17 (0.99-1.45), p=0.06. Of interest, patients commenced on zidovudine (n=1364) were not at a higher risk of developing a worsening of anaemia compared with those initiated on stavudine (n=1579), HR (95% CI) of 0.90 (0.75-1.07), p=0.23. In a survival analysis using Kaplan-Meier methodology which took into account censoring due to discontinuation of NNRTI for any reason, 321 (18.1%) patients on efavirenz-based cART (n=1769) compared with 196 (16.7%) patients on nevirapine-based cART (n= 1174) experienced at least one episode of severe to life-threatening anaemia giving an unadjusted HR (95% CI) of 1.11 (0.93-1.35), p=0.22. The factors which remained significant in the adjusted analysis was low BMI<18.5 kg/m^2 and having AIDS diagnosis at baseline.

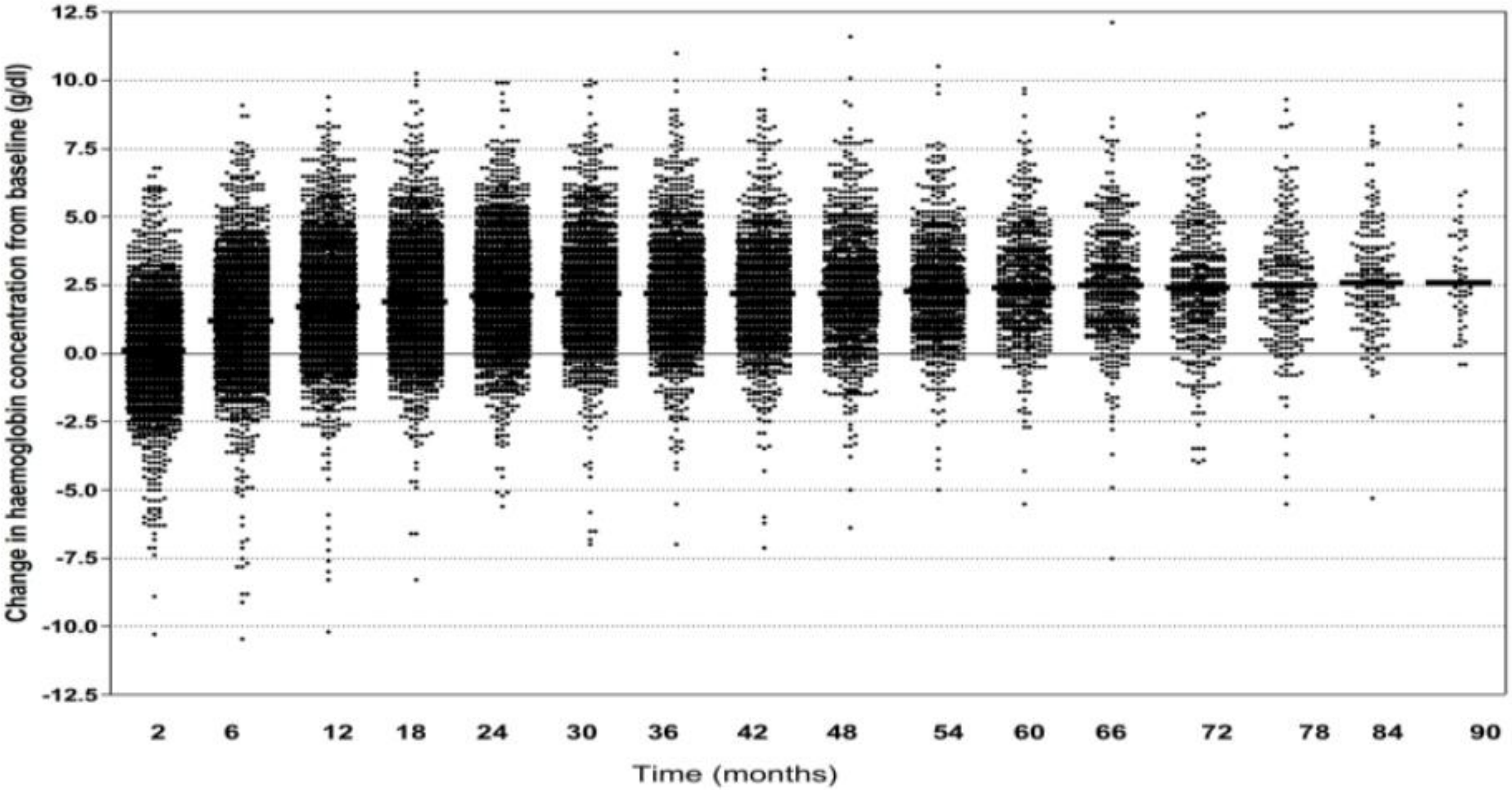


Figure 5.1. Changes in haemoglobin concentration from baseline in patients with some degree of anaemia at initiation of cART. Each dot represents a subject and the horizontal lines are the median of haemoglobin concentration change.

(iii) Model for predictive factors associated with the risk of developing severe anaemia among patients without anaemia at the start of cART.

Four hundred and seventy-seven (477) patients included in this sub-analysis initiated cART without anaemia. 358 (75.0%) patients in this subset of patients experienced some degree of anaemia with only 21 (4.4%) experiencing at least one episode of severe anaemia. Recurrent anaemia of more than one episode occurred in 248 (52.0%) patients under follow-up with a range of 2 to 15 episodes per patient.

On adjusted multivariate analyses, females had a 20% lower risk of developing any degree of anaemia compared with males (HR 0.80; 95% CI 0.46-0.96; p=0.03), and patients on zidovudine had a 31% higher risk of anaemia compared with those on stavudine (HR 1.31; 95% CI 1.14-2.02; p=0.004). There was no significant difference in the risk of developing anaemia on efavirenz (HR 1.14; 95% CI 0.94-1.62; p=0.13) compared with nevirapine. Also, of the 21 patients who experienced severe anaemia in this sub-category of study subjects, all 21 patients were females, 17 were on zidovudine vs 4 on stavudine and 14 were nevirapine vs 7 on efavirenz. The small number of clinical events limited the ability to perform further analysis. Evidence of haemoglobinopathies, parasitic infestations and other causes of anaemia such as heavy menstrual blood loss in females were not recorded in patient database and therefore could not be accounted for in these analyses.

Treatment-limiting toxicity: Out of the 675 episodes of severe anaemia, only 62 (9%) events - all among patients on zidovudine-containing regimens - were considered severe enough to warrant therapy change to stavudine.

(2) Incidence and risk factors for developing skin rash on cART

Incidence rates of skin rash: There were 341 cases of skin rash recorded while patients were on ART giving an incidence rate of 3.03 events per 100 person-years of follow up. 295 patients had one episode of skin rash, 21 patients had 2 episodes and 1 patient had 4 episodes of skin rash on follow up. The median time to onset of first episode of all skin rash was 2 months (range 2-48 months). 299 events of skin rash were NNRTI-related while 42 were not NNRTI-related. Reasons other than NNRTI-associated skin rash were HIV-associated nodular prurigo (n=28), pruritic papular dermatitis (n=7), varicella zoster rash (n=3), septrin rash (n=2), tinea corporis (n=1), abacavir hypersensitivity rash (n=1).

180 out of 341 (53%) skin rashes occurred in patients on nevirapine-based ART while 158 out of 341 (46%) skin rashes occurred in patients on efavirenz-based ART and 3 (1%) occurred in patients in patients on ritonavir-boosted lopinavir-based ART. The odds ratio of developing any skin rash on a nevirapine-based ART compared with efavirenz-based ART was 1.75 (1.40 – 2.19), $p<0.0001$.

Incidence of NNRTI-related skin rash: 281 (7.0% of 3,999 patients who started NNRTI) patients experienced 299 events of NNRTI-related rash, 264 patients had one episode and 17 had more than one episode of rash: 16 had 2 episodes and one patient had 3 episodes of mild grade 1 rash at months 2, 6, and 12. The frequency of nevirapine-associated rash was 10.2% (n=1,623) while efavirenz-associated rash was 5.6% (n=2,376). Table 5.3 shows the frequencies of the various grades of NNRTI-related skin rash. 90 (30%) of the NNRTI skin rash were grade I, 70 (23%) were grade IIA, 122 (41%) were grade IIB, 15 (5%) were grade III and 2 (1%) were grade IV. Grade III skin rash included Stevens Johnson syndrome (n=7), generalised maculopapular rash with constitutional symptoms such as fever and malaise (n=5), and maculopapular rash with hepatotoxicity (n=3). The frequency of severe to life-threatening skin rash on nevirapine was 0.7% (n=1,623) and on efavirenz was 0.2% (n=2,376). There were no significant differences in the severity of NNRTI-related skin rash although grade 3 and 4 skin rash were commoner among patients on nevirapine-based ART compared with those on efavirenz-based ART.

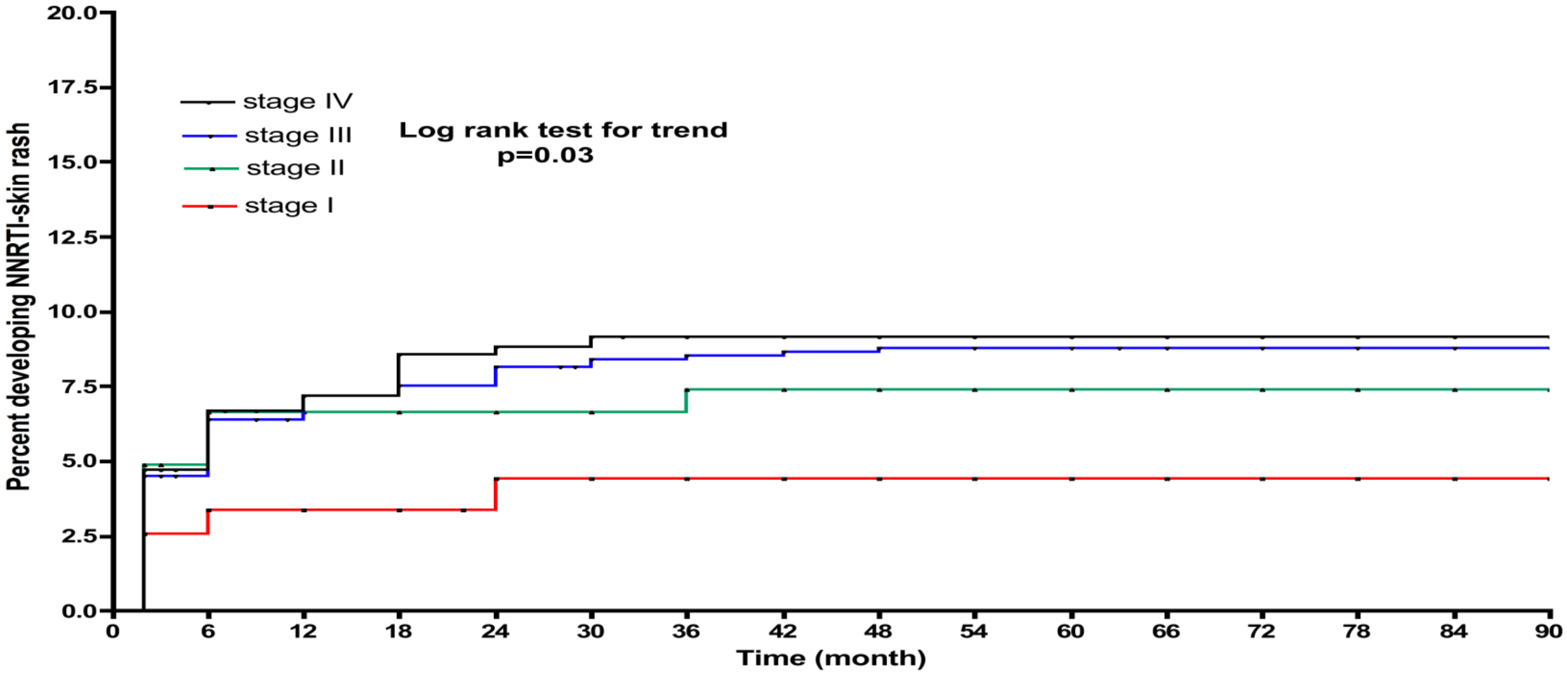
Table 5.3: Severity of NNRTI-related skin rash among Ghanaian HIV patients

Type	Nevirapine n=165 (%)	Efavirenz n=134 (%)	Total n=299 (%)	Chi-square test p-value
I	44 (27%)	46 (34%)	90 (30%)	0.13
IIA	43 (26%)	27 (20%)	70 (23%)	0.27
IIB	66 (40%)	56 (42%)	122 (41%)	0.80
III	10 (6%)	5 (4%)	15 (5%)	0.35
IV	2 (1%)	0 (0%)	2 (1%)	0.20

Grade I –erythema/hyperpigmented rash localised, Grade IIA – diffuse maculopapular rash, Grade IIB- urticaria, Grade III- grade I,II + constitutional symptoms or angioedema or serum sickness-like reaction or Stevens Johnson Syndrome. Grade IV- toxic epidermal necrolysis.

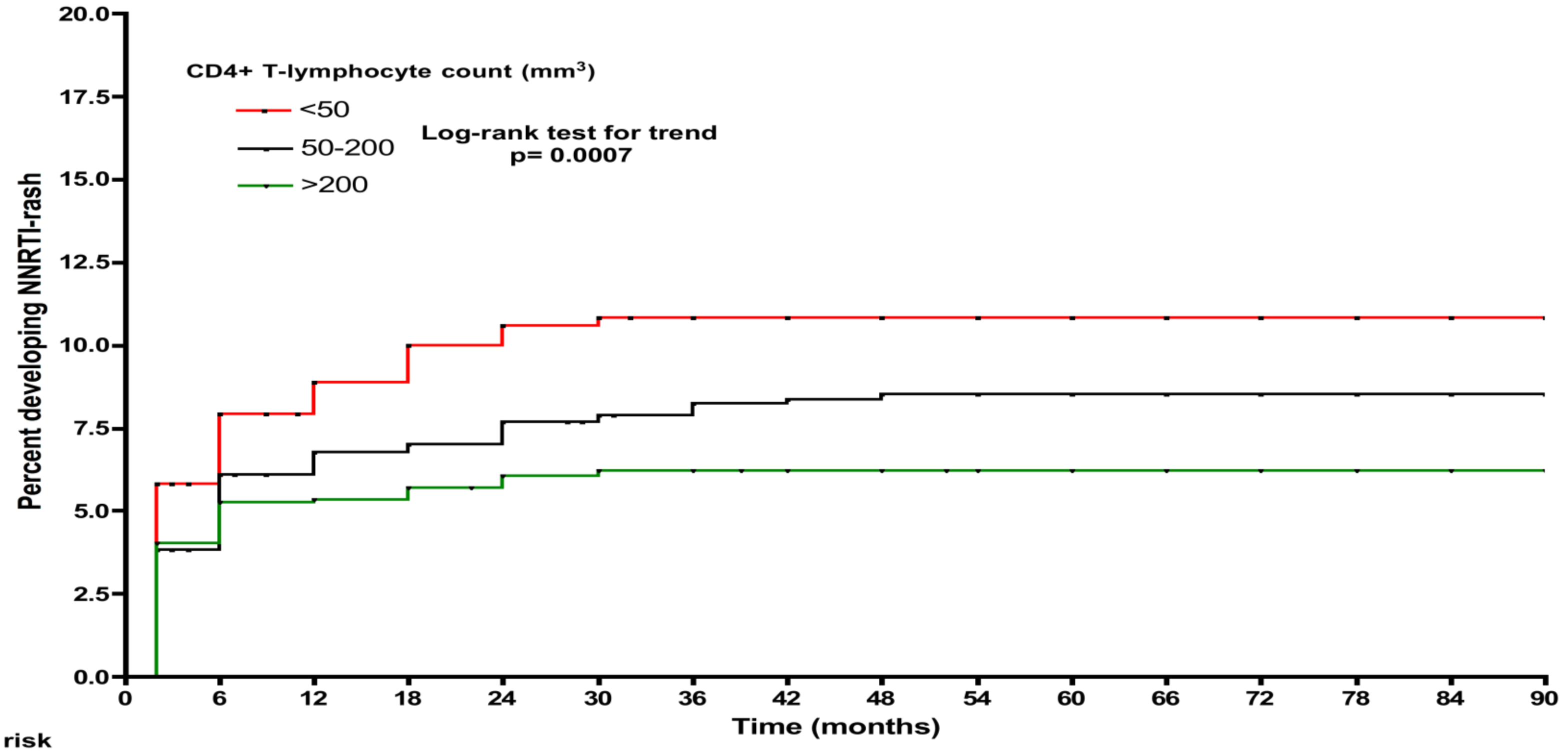
Risk factors for NNRTI-related skin rash: Use of nevirapine based ART was associated with an unadjusted hazard ratio (95%CI) of 1.83 (1.48 – 2.41), p<0.0001 of developing an NNRTI-related skin rash compared with use of an efavirenz based ART. Other baseline risk factors significantly associated with NNRTI-related skin rash on univariate analyses were female gender with HR of 1.57 (1.18 – 1.97), low body mass index, advanced WHO clinical stage (Figure 5.2), low CD4 (Figure 5.3) and hepatitis B virus seropositivity as shown in Table 5.4. Age, ALT and AST baseline concentrations were not associated with risk of NNRTI-related skin rash. In a Cox proportional hazards multivariate model, use of NVP was associated with an adjusted HR (95%CI) of 1.67 (1.28 – 2.10), p=0.0002. Other factors which remained significantly associated with risk of developing NNRTI-related skin rash included female gender HR (95% CI) of 1.39 (1.01 – 1.92), p=0.04 and CD4 counts with HR (95%CI) of 0.88 (0.82 – 0.95) p=0.0005 with each 50 cells increment in baseline CD4. Although HBV seropositivity as associated with risk of NNRTI-related skin rash in the univariate model, this factor was not included in the final baseline multivariate model (n=3417) because the number of observations in this variable limited the power of this model.

Immunological basis for NNRTI-related skin rash?: Profound increases in CD4 T-cell counts during the initial phases of treatment has been proposed as one of the possible explanations for the development of NNRTI-skin rash[18,19]. Given that the risk for developing skin rash was significantly higher for those with low CD4 counts at baseline, a comparison of the percentage and absolute changes in CD4 counts within the first 12 months of therapy was performed among those who developed NNRTI-skin rash and those who did not. The median (IQR) of the percentage change in CD4+ T-cell counts at 6 months among patients who developed NNRTI-skin rash was +190% (+93% to +576%, n=219) compared with +119% (+57% to +272.5%, n=2512), p<0.0001 for those without skin rash and +250% (+103% to +724%, n=229) versus +143% (+71% to 325.5%, n=2471) p<0.0001, respectively at 12 months. The median (IQR) of the absolute change in CD4+ T-cell counts from baseline among patients who developed NNRTI-skin rash compared with those did not develop this event at 6 and 12 months were 172 (108-283/mm^3) vs 159 (85-248/mm^3) p=0.02 and 237 (147.5-335.5/mm^3) vs 199 (114-304/mm^3) p=0.0008 respectively. Thus compared with those who had no skin rash, patients who developed NNRTI-related skin rash had more profound increases in CD4 counts from baseline. Changes in the median ALT and AST concentration at months 2, 6 and 12 between those with or without NNRTI-rash were not significantly different (data not shown).



Number at risk										
Stage I	273	253	232	185	106	67	50	40	18	4
Stage II	476	427	393	332	242	171	112	84	32	7
Stage III	2068	1728	1542	1282	888	651	415	313	134	46
Stage IV	584	437	384	317	235	188	104	77	37	8

Figure 5.2. Kaplan-Meier estimates of risk for NNRTI-related skin rash according to WHO clinical stage at baseline.



Number at risk

CD4<50	909	673	573	461	313	210	139	118	47	13
CD4 50-200	1708	1464	1326	1109	802	601	365	277	125	37
CD4 >200	1203	1081	992	781	473	341	204	141	57	18

Figure 5.3. Kaplan-Meier estimates of risk for NNRTI-related skin rash according to CD4 count at baseline.

Table 5.4. Baseline risk factors associated with NNRTI-related skin rash among Ghanaian HIV patients on cART.

Variable	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
NNRTI				
Nevirapine	1.83 (1.48 – 2.41)	<0.0001	1.67 (1.28 – 2.19)	0.0002
Efavirenz	1.00		1.00	
Gender				
Female	1.57 (1.18 – 1.97)	0.0013	1.39 (1.01 – 1.92)	0.04
Male	1.00		1.00	
Age				
<40 years	1.34 (1.06 – 1.71)	0.01	1.11 (0.85-1.45)	0.45
≥ 40 years	1.00		1.00	
BMI per 5kg/m^2 increase				
	0.86 (0.73 – 0.99)	0.05	0.94 (0.79 – 1.11)	0.47
Clinical stage per each WHO stage higher				
	1.21 (1.02 – 1.42)	0.03	1.13 (0.94 – 1.35)	0.20
CD4 counts per 50 cells increase				
	0.89 (0.83 – 0.94)	0.0001	0.88 (0.82 – 0.95)	0.0005
HBV status				
positive	1.48 (1.03 – 2.49)	0.04	Not included*	-
negative	1.00			
ALT per 10IU/l higher				
	1.01 (0.99 – 1.04)	0.33	-	-

* Not included number of patients whose HBV sero-status was small.

NVP-rash versus EFV-rash: Although the risk factors for NVP-rash have been well characterised, those for EFV-rash still remains to be elucidated. Thus, an analysis was performed to identify whether risk factors predisposing to NVP-rash were different from those of EFV-rash. First, the median (range) time to the first reported rash was not significantly different for NVP-rash 2 months (2-36) compared with EFV-rash 2 months (2-48), p=0.12. Second, both NVP-rash and EFV-rash shared a predilection for occurring among patients starting with a low CD4 count with the adjusted HRs of developing NVP-rash or EFV-rash with CD4 counts below 100 cell/mm^3 of 1.48 (1.09 – 2.03), p=0.01 and 1.61 (1.09 – 2.39), p=0.02 respectively. Third, significant differences in associations were not found in the risk for either specific NNRTI-rash in relation to baseline age, NRTI backbone upon which the NNRTI is started on, or ALT/AST concentrations either at baseline or their changes at 2, 6 and 12 months on treatment (data not shown). Thus although NVP-rash is more frequent than EFV-rash, both appear to share common risk factors in this cohort.

Treatment-limiting toxicity Forty-four (44) cases of NNRTI-related rash were treatment-limiting requiring modifications in therapy. These included 2 cases of grade IV skin rash, 10 cases of grade III, 16 cases of grade IIB skin rash and 16 cases of grade IIA. These treatment-limiting adverse events were observed in 41 out of 165 (25%) patients on NVP compared with 3 out of 134 (2%) on EFV giving rise to a relative risk ratio (95% CI) of 11.10 (3.51 – 35.06), p<0.0001. Among the 41 NVP-treatment-limiting skin rash, 39 were switched to EFV-based ART, 1 to nelfinavir-based ART and 1 to ritonavir-boosted lopinavir. Of the 3 cases of EFV-associated skin rash one was changed to ritonavir-boosted lopinavir and two others to nelfinavir. As mentioned in chapter 4, two patients died from nevirapine-associated Stevens Johnsons' syndrome.

Cross-reactivity cutaneous reaction: There were 4 probable cases of cross-reactivity cutaneous reactions after substituting one NNRTI for the other. The baseline characteristics, cART regimens, description of the first and second rash and outcomes are as shown in Table 5.5 below. All four cases involved a substitution for efavirenz on account of nevirapine-associated rash. These rashes resolved initially upon substituting nevirapine but patients experienced another on efavirenz rash thought to be NNRTI-related. However, these rashes resolved without any further recourse to further substitutions of NNRTI with one patient requiring an antihistamine for symptomatic management of pruritus.

Table 5.5. Four cases of probable cross-reactive cutaneous reactions due to NNRTI class substitutions

Gender	Age	WHO clinical stage	CD4 count mm^3	cART regimen	Time to 1st rash (months)	Description and stage of 1st rash	cART regimen changed to	Time to 2nd rash months	Description and stage of 2nd rash	Outcome of 2nd rash
Female	39	4	26	D4T+3TC+NVP	2	Diffuse pruritic rash, IIB	D4T+3TC+EFV	6	Hyperpigmented rash, I	Resolved without any intervention
Male	40	Not recorded	210	AZT+3TC+NVP	2	Generalised maculo-papular rash, IIA	AZT+3TC+EFV	6	Hyperpigmented, pruritic rash, I	Resolved without any intervention
Female	52	3	7	D4T+3TC+NVP	2	Generalised maculo-papular rash with mucosal involvement and ulceration, IV	D4T+3TC+EFV	6	Hyperpigmented rash, I	Resolved without any intervention
Female	34	3	56	AZT+3TC+NVP	2	Generalised maculopapular rash, IIA	AZT+3TC+EFV	12	Generalised pruritic rash, III	Resolved on antihistamines

(3) Incidence and risk factors for neuro-psychiatric toxicity on ART

Incidence rates: 218 patients experienced a total of 235 events of neuropsychiatric toxicities during follow up. 203 patients had one event, 13 patients experienced 2 events and 2 patients experienced 3 episodes of neuropsychiatric events during follow up. Thus the frequency of reported neuropsychiatric toxicity was 5.5% (n=3,999) with 7.6% (n=2,376) of efavirenz recipients and 2.4% (n=1,623) of nevirapine recipients experiencing this adverse event. The median (range) time to first episode of neuropsychiatric toxicity was 2 months (2-84 months). Most neuropsychiatric toxicities were reported in the first year of therapy after which incidence of events decline as shown in Figure 5.4. 195 events were reported among patients on efavirenz-based ART compared to 40 events among those on nevirapine-based ART, odds ratio of 3.54 (2.50 – 5.00), p<0.0001. A total of 252 neuropsychiatric symptoms were reported, the commonest being insomnia (n=126), headaches (n=19), dizziness (n=17), abnormal dreams (n=14) and drowsiness (13) are shown in Table 5.6. Insomnia, headaches, abnormal dreams and drowsiness were significantly commoner among patients on EFV-based ART compared with NVP-based ART. Other notable but rarely reported NNRTI neuropsychiatric toxicity includes cerebellar ataxia (n=2), dysarthria (n=5) and seizures (n=3).

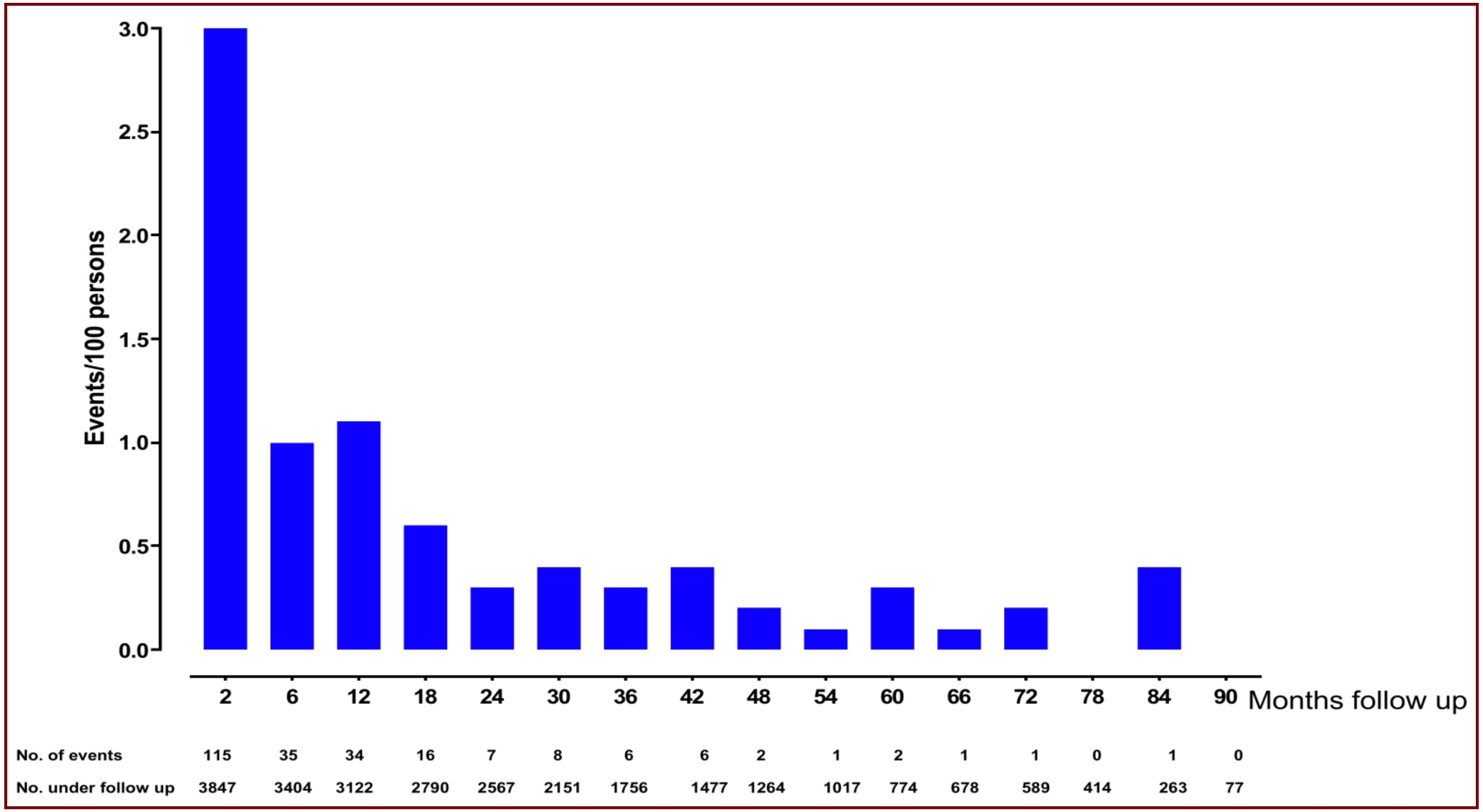


Figure 5.4. A graph showing the incidence rates of neuropsychiatric symptoms attributed to toxicity over follow-up on ART among Ghanaian HIV-infected patients.

Table 5.6. Frequency of neuro-psychiatric side effects of ART among Ghanaian HIV patients

Neuro-psychiatric toxicity	Patients on efavirenz with toxicity n=208 (%)	Patients on nevirapine with toxicity n=44 (%)	p-value	Total number of events n=252 (%)
Insomnia	103 (49.5)	23 (52.3)	<0.0001	126 (50.0)
Headaches	16 (7.7)	3 (6.8)	0.05	19 (7.5)
Dizziness	12 (5.8)	5 (11.4)	0.49	17 (6.7)
Abnormal dreams	13 (6.3)	1 (2.3)	0.02	14 (5.6)
Drowsiness	13 (6.3)	0 (0.0)	0.007	13 (5.2)
Aggressive behaviour	8 (3.8)	2 (4.5)	0.31	10 (4.0)
Incoherent speech	6 (2.9)	1 (2.3)	0.30	7 (2.8)
Myalgia	3 (1.4)	4 (9.1)	0.61	7 (2.8)
Dysarthria	4 (1.9)	1 (2.3)	0.63	5 (2.0)
Dystonia	5 (2.4)	0 (0.0)	0.16	5 (2.0)
Mood changes	5 (2.4)	0 (0.0)	0.16	5 (2.0)
Tremors	2 (1.0)	3 (6.8)	0.67	5 (2.0)
Confusion	3 (1.4)	0 (0.0)	0.40	3 (1.2)
Seizures	3 (1.4)	0 (0.0)	0.40	3 (1.2)
Cerebellar ataxia	2 (1.0)	0 (0.0)	0.65	2 (0.8)
Psychosis	2 (1.0)	0 (0.0)	0.65	2 (0.8)
Somnolence	1 (0.5)	1 (2.3)	0.65	2 (0.8)
Vertigo	2 (1.0)	0 (0.0)	0.65	2 (0.8)
Anxiety	1 (0.5)	0 (0.0)	0.85	1 (0.4)
Erectile dysfunction	1 (0.5)	0 (0.0)	0.85	1 (0.4)
Hyperactivity	1 (0.5)	0 (0.0)	0.85	1 (0.4)
Impaired concentration	1 (0.5)	0 (0.0)	0.85	1 (0.4)
Suicidal ideation and attempt	1 (0.5)	0 (0.0)	0.85	1 (0.4)
TOTAL	208	44	<0.0001	252

Risk factors for neuropsychiatric toxicity: Predictor variables included in univariate analysis of risk factors for neuro-psychiatric toxicity included gender, age, BMI, CD4 cell count at baseline, WHO clinical stage, HBV sero-status and NNRTI on which patient developed toxicity. As shown in Table 5.7, the only factors identified to be associated with neuro-psychiatric toxicity included age ≥35 years at initiation of therapy with HR (95% CI) of 1.55 (1.16 – 2.00), p=0.003, BMI<16kg/m^2 with HR (95% CI) of 1.45 (1.03 – 2.04), p=0.04 and use of efavirenz with HR (95% CI) of 3.43 (2.16 – 3.72), p<0.0001. Furthermore on multivariate analysis, BMI<16kg/m^2 and use of efavirenz were significantly associated with risk of developing neuro-psychiatric toxicity on ART among Ghanaian HIV-infected patients with HR (95% CI) of 1.44 (1.02 – 2.03),p=0.04 and 3.29 (2.32 – 4.69),p<0.0001 respectively.

Treatment limiting adverse toxicity: 33 of 195 events (17%) among patients on EFV-based ART led to substitution of efavirenz by nevirapine while 6 of 40 (15%) events among patients on NVP-based ART led to substitution of nevirapine by efavirenz (n=5) and nelfinavir (n=1). The substitutions of nevirapine by efavirenz on account of neuro-psychiatric events were performed in 5 patients because they were to initiate anti-tuberculous therapy at the time when those toxicities occurred on nevirapine. This was to avoid drug interactions between nevirapine and rifampicin-which is used as a component of the quadruple antituberculous drug regimen. However, most neuropsychiatric symptoms resolved under continual therapy without having to alter NNRTI on account of neuro-psychiatric toxicity.

Table 5.7. Risk factors associated with developing neuro-psychiatric toxicity among Ghanaians HIV patients

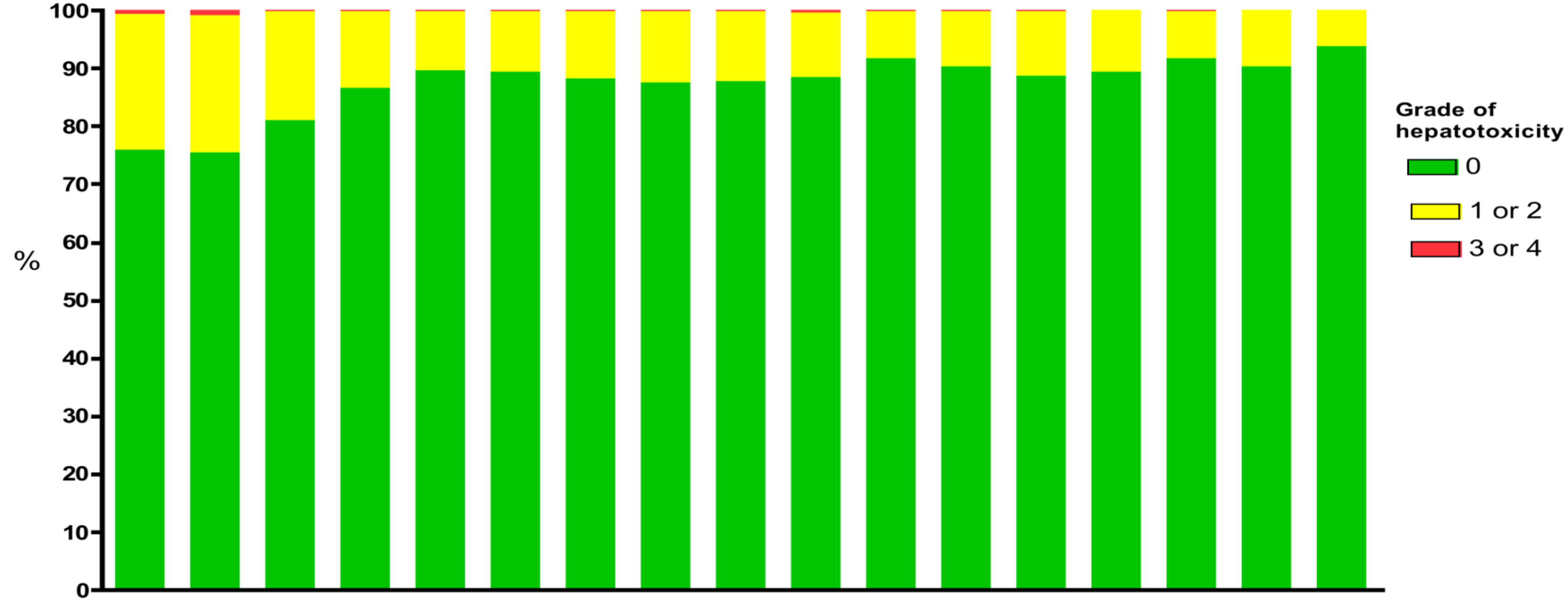
Predictor	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Gender				
Male	0.87 (0.65 – 1.15)	0.32	-	-
Female	1.00			
Age				
≥35 years	1.55 (1.16 – 2.00)	0.003	1.22 (0.90 – 1.64)	0.19
<35 years	1.00		1.00	
BMI				
<16kg/m^2	1.45 (1.03 – 2.04)	0.04	1.44 (1.02 – 2.03)	0.04
≥ 16kg/m^2	1.00		1.00	
WHO clinical stage				
stage 3 or 4	0.91 (0.65 – 1.28)	0.60	-	-
stage 1 or 2	1.00			
Baseline CD4 counts				
<200	0.97 (0.72 – 1.30)	0.83	-	-
≥200	1.00			
HBV status				
positive	1.53 (0.98 – 2.39)	0.06*	-	-
negative	1.00			
NNRTI				
Efavirenz	3.43 (2.16 – 3.72)	<0.0001	3.29 (2.32 – 4.69)	<0.0001
Nevirapine	1.00		1.00	

* Not included number of patients whose HBV sero-status was small.

(4) Incidence and risk factors for developing severe hepatotoxicity on ART

Incidence rates: Before treatment there were 3702 measurements of ALT for 4039 patients (76%) of which 2813 (76.0%) had normal ALT levels, 865 (23.4%) had grade 1 or 2 hepatotoxicity and 24 (0.6%) had grade 3 or 4 hepatotoxicity. For patients who remained on therapy during follow up, there was a general increase in the proportion of patients with normal ALT levels up to 93% at 90 months follow up as shown in Figure 5.5.

Overall, 143 patients experienced severe hepatotoxicity defined as elevated serum ALT levels ≥5 times the upper limit of normal or elevation of ALT by more than 100IU/l above baseline concentration during 11,236.8 person-years of follow up with an incidence rate of 1.27/100 person-years, 95%CI of 1.07 – 1.49/100 person years. 131 patients experienced one episode, 9 experienced 2 episodes, 2 experienced 3 episodes and 1 experienced 5 episodes of severe hepatotoxicity during follow-up giving a total of 160 events with an event rate of 1.42/100 person years. The median (range) time to the onset of first episode of severe hepatotoxicity was 12 months (2-84). Overall, the frequency of severe hepatotoxicity was 3.9% (143 patients of 3702 evaluable with ALT measurements at baseline). By Kaplan Meier methodology, the estimated percent cumulative incidence (95% CI) of severe hepatotoxicity at 12, 36, 72 and 90 months were 2.3% (1.8% - 2.8%), 3.5% (2.8% - 4.2%), 5.6% (4.4% - 6.8%) and 6.7% (5.1% - 8.3%) respectively.



Months follow up	0	2	6	12	18	24	30	36	42	48	54	60	66	72	78	84	90
No. of observations	n= 3702	2096	2070	2063	1875	1771	1452	1205	1061	919	713	564	514	472	320	206	63
Grade of hepatoxicity																	
0	n= 2813	1581	1679	1786	1680	1583	1282	1053	930	812	653	509	456	422	293	186	59
1 or 2	n= 865	496	384	271	191	183	166	149	129	103	58	54	57	50	26	20	4
3 or 4	n= 24	19	7	6	4	5	4	3	2	4	2	1	1	0	1	0	0

Figure 5.5. Proportion of patients with grades of hepatotoxicity before and during cART among Ghanaian HIV-infected patients. Grade 0- ALT within ULN, Grade 1- ALT 1.1-2.5 ULN, Grade 2- ALT 2.6 – 5.0ULN, Grade 3- ALT 5.1 – 10.0 ULN, Grade 4- ALT>10.0 ULN. Grade 3 or 4 are regarded as severe hepatotoxicity.

The frequencies of severe hepatotoxicity among efavirenz and nevirapine recipients were 3.7% (78 of 2376 patients who started EFV) and 3.8% (62 of 1,623 who started on NVP). The median time of first episode of severe hepatotoxicity among NVP-recipients of 6 months (range of 2 to 84 months) was significantly earlier than those among EFV-recipients of 12 months (range of 2 to 78 months), p=0.03.

Risk factors: Hepatitis B sero-positivity was significantly associated with risk of developing severe hepatotoxicity with a HR (95%CI) of 1.99 (1.16 – 3.40), p=0.01 shown in Figure 5.6. The only other significant risk factor identified in univariate analyses was baseline body mass index with an HR (95% CI) of 1.21 (1.00 – 1.47) for each 5kg/m^2 increase in BMI. Factors such as age, gender, WHO clinical stage, CD4 counts at baseline and its changes within first 12 months of ART, NNRTI and NRTI used were all not associated with risk of developing severe hepatotoxicity. Changes in BMI during therapy was not associated with risk of hepatotoxicity (data not shown). On multivariate analysis, the only factor significantly associated with risk of severe hepatotoxicity was hepatitis B sero-positivity as shown in Table 6.8. The cumulative frequency of severe hepatotoxicity by Kaplan Meier estimates among HBV sero-positive patients during follow up was 12.9% (95% CI of 5.9% to 20.0%) compared to 6.7% (95% CI of 4.3% - 9.0%) among HBV sero-negative patients. Among HBV sero-positive patients, the cumulative frequency among efavirenz recipients of 12.5% was not significantly different from those on nevirapine of 12.0%.

Of the 143 events of severe hepatoxicity, 83 events occurred in patients on efavirenz-based ART, 56 events occurred in patients on nevirapine-based ART and 4 events on a protease inhibitor-based ART (3 on ritonavir-boosted lopinavir and 1 on nelfinavir).

Treatment limiting toxicity: Only 8 of these events, all in patients on NVP-based ART were considered treatment-limiting requiring substitution with efavirenz (n=8).

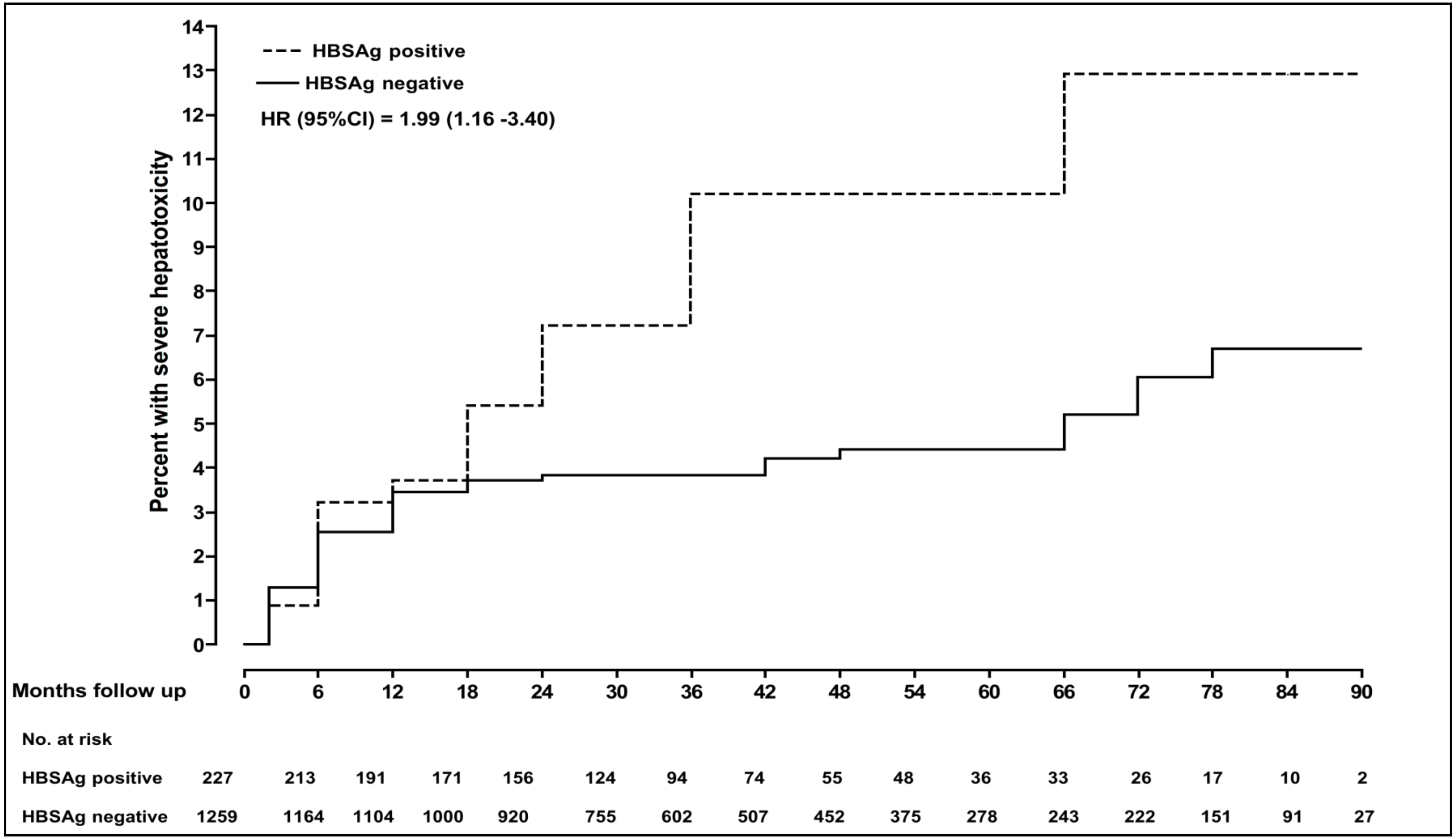


Figure 5.6. Kaplan-Meier survival analysis of risk of severe hepatotoxicity on NNRTI-based ART according to HBSAg sero-status among Ghanaian HIV patients. HBSAg= Hepatitis B Surface Antigen, HR=hazard ratio.

Table 5.8. Risk factors of severe hepatotoxicity on ART among Ghanaian HIV-infected patients

Predictor	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Gender				
Female	0.83 (0.59 – 1.18)	0.31	-	-
Male	1.00			
Age per 10 year increase				
	0.85 (0.71 – 1.02)	0.08	-	-
BMI per 5kg/m^2 increase				
	1.21 (1.00 – 1.47)	0.04	1.02 (0.77 – 1.36)	0.89
WHO clinical stage				
stage 3 or 4	1.46 (0.92 -2.31)	0.11	-	-
stage 1 or 2	1.00			
Baseline CD4 counts per 50 cells increase				
	1.02 (0.95 – 1.11)	0.54	-	-
HBV status				
positive	1.99 (1.16 – 3.40)	0.01	1.99 (1.16 -3.40)	0.01
negative	1.00			
NNRTI				
Nevirapine	1.04 (0.74 – 1.45)	0.84	-	-
Efavirenz	1.00			
NRTI				
Stavidine	1.02 (0.96 – 1.09)	0.48	-	-
Zidovudine	1.00			
CD4 change in 6 months per each 50 cells increase	0.99 (0.92 – 1.06)	0.68	-	-
CD4 change in 12 months per each 50 cells increase	0.99 (0.93 – 1.04)	0.62	-	-

(5) Incidence and risk factors for NRTI mitochondria-related ART toxicities among Ghanaian HIV infected patients

Incidence rates: 222 patients reported experiencing at least one episode of NRTI mitochondria-related ART toxicity during follow up. These included peripheral neuropathy (n=181), lipoatrophy (n=40), lactic acidosis (n=4) and pancreatitis (n=1). Overall the median time to onset of first reported mitochondria-related toxicity is 12 months (range of 2-72 months), with the median time of onset of first episode of peripheral neuropathy and lipoatrophy of 6 months (range of 2-72 months) and 42 months (range of 2-66 months) respectively. 193 of the mitochondrial-related toxicity occurred among patients on stavudine, 27 occurred among patients on zidovudine, 1 patient on didanosine and 1 patient on tenofovir.

The percent cumulative incidence (95% CI) by Kaplan-Meier estimates of peripheral neuropathy at 12, 36, and 72 months were 3.4% (2.8% - 4.0%), 5.7% (4.8% - 6.5%) and 6.7% (5.6% - 7.8%) respectively. The percent cumulative frequencies specifically among recipients of stavudine at 12, 36 and 66 months were 5.6% (4.5% - 6.7%), 9.6% (8.1% - 11.2%) and 11.3% (9.3% -1 3.2%) respectively. The cumulative incidence of lipoatrophy among patients on stavudine at 12, 36 and 66 months were 0.1% (0% - 0.2%), 1.6% (0.8% - 2.4%) and 6.5% (4.3% - 8.8%) respectively.

Risk factors for mitochondria-associated toxicity: The risk factors identified for NRTI mitochondria-related toxicity on univariate analysis with their associated HR (95% CI) and p-values are as follows: female gender 1.47 (1.08 – 2.00), p=0.02; age above 40 years at initiation of therapy-1.98 (1.51-2.59), p<0.0001; initiating therapy with clinical stages III or IV disease compared with stages I or II- 1.52 (1.05 – 2.19), p=0.03;

baseline haemoglobin concentration below 8g/dl-1.98 (1.44 - 2.73), p<0.0001; baseline BMI below 18.5kg/m^2-1.44 (1.10 – 1.88),p=0.008 and initiating stavudine-containing ART compared with zidovudine-containing ART-7.80 (5.21 – 11.67), p<0.0001. Factors not significantly associated with NRTI mitochondria-related toxicity included baseline CD4 cell count strata of below 200 cells/mm^3 vs ≥200 cells/mm^3, baseline eGFR below 60ml/min vs ≥60ml/min and NNRTI used to initiate therapy efavirenz vs nevirapine. On multivariate analysis, the only factors that were significantly associated with risk of developing NRTI mitochondria-related toxicity included female gender adjusted HR of 1.40 (95% CI of 1.00-1.96), age above 40 years at initiation of therapy with an adjusted HR of 2.08 (95% CI of 1.55-2.79) and stavudine-containing regimen with an adjusted HR of 7.09 (95% CI of 4.62- 10.89).

Risk factors for peripheral neuropathy: As shown in Table 6.9, the risk factors for developing peripheral neuropathy on univariate analysis included age ≥40 years, WHO stages III or IV at initiation of ART, baseline haemoglobin concentration below 8g/dl, baseline BMI below 18.5kg/m^2 and starting a stavudine-containing regimen. Age ≥40 years and starting a stavudine-containing regimen remaining significant risk factors on multivariate analysis.

Table 5.9. Risk factors for developing peripheral neuropathy on ART among Ghanaian HIV infected patients

Predictor	Unadjusted HR (95% CI)	p-value	Adjusted HR (95% CI)	p-value
Gender				
Female	1.35 (0.96 – 1.89)	0.08	-	-
Male	1.00			
Age				
≥40 years	2.09 (1.54 – 2.83)	<0.0001	2.21 (1.59 – 3.07)	<0.0001
<40 years	1.00		1.00	
BMI				
<18.5 kg/m^2	1.35 (1.00 – 1.82)	0.05	0.90 (0.65 – 1.26)	0.56
≥ 18.5 kg/m^2	1.00		1.00	
WHO clinical stage				
stage 3 or 4	1.63 (1.07 – 2.48)	0.02	1.31 (0.85 – 2.01)	0.22
stage 1 or 2	1.00		1.00	
Baseline CD4 counts				
<200 cells/mm^3	1.15 (0.83 – 1.57)	0.40	-	-
≥200 cells/mm^3	1.00			
Baseline HB				
<8.0g/dl	1.65 (1.14 – 2.39)	0.008	0.98 (0.65 – 1.47)	0.92
≥ 8.0g/dl	1.00		1.00	
eGFR				
<60ml/min	1.06 (0.72 – 1.55)	0.77	-	-
≥60ml/min	1.00			
NRTI				
Stavudine	6.13 (4.04 – 9.29)	<0.0001	5.63 (3.64 – 8.70)	<0.0001
Zidovudine	1.00		1.00	
NNRTI				
Efavirenz	1.04 (0.77 – 1.40)	0.80	-	-
Nevirapine	1.00			

Risk factors for lipoatrophy: Because lipoatrophy was reported exclusively among patients on stavudine-containing regimen, risk factors analysis was performed among patients who either initiated a d4T-containing ART regimen or were switched to a stavudine-regimen during follow up. The risk factors for developing stavuudine-associated lipoatrophy on univariate analysis were female gender and starting stavudine with baseline haemoglobin concentration below 8g/dl with only baseline haemoglobin concentration below 8g/dl as the only significant risk factor for developing stavudine-associated lipoatrophy with an adjusted HR of 2.75 (1.37 – 5.53), p=0.005.

Probable lactic acidosis: There were 4 documented cases of probable lactic acidosis all among recipients of stavudine which culminated in deaths as recorded in chapter 4. A descriptive account of one such case is given below: The patient was a 42-year-old female who started an ART regimen comprising stavudine plus lamivudine with nevirapine on 22/08/2008. At the time of treatment initiation, her haemoglobin concentration was 7.1g/dl, had a WHO clinical stage III disease with a CD4 count of 182 cells/mm^3 and had BMI of 14.0kg/m^2. In the sixth month of follow up, the patient presented with general malaise, bodily weakness, vaguely localising abdominal pain and hepatomegaly and in shock. She was admitted and a clinical diagnosis of lactic acidosis probable from stavudine being made and resuscitated but died within 3 days after her admission.

Treatment-limiting toxicity: Of the 181 recorded cases of peripheral neuropathy on ART, 152 (84%) were on stavudine-containing regimen, 26 (14%) were on zidovudine-containing regimen and the rest were on didanosine-containing regimen. 83 events of peripheral neuropathy (46%) all among patients on a stavudine-containing regimen led to drug substitutions predominantly for zidovudine (n=81) and 2 to tenofovir. Only 19 events led to immediate drug substitutions at time of first report of peripheral neuropathy, the remainder had a median time lag to substitution of 6 months (range 4-70 months).

Thirty-four (34) out of 40 reported cases of lipoatrophy led to substitution of stavudine for zidovudine (n=31), tenofovir (n=2) and abacavir (n=1). In 19 patients substitutions were effected upon first report of lipoatrophy by patients, while delays of up to 6 months (n=6), 12 months (n=1), 16 months (n=1), 42 months (n=1) were observed before switching. Of note, in 6 patients a switch to zidovudine at 6 months (n=4) and 12 months (n=2) was performed to prevent lipoatrophy but patients eventually developed this toxicity in spite of this substitution.

(6) Impact of ART-associated toxicity on adherence

Table 5.10 shows results of a multiple logistic regression analysis of associations between the occurrence of specific and all-cause toxicity and the risk of non-adherence. With the exception of developing mitochondrial toxicity, ART-specific toxicities such as developing severe anaemia, severe hepatotoxicity, neuropsychiatric toxicity, skin rash and all-cause toxicity were all significantly associated with the risk of reported non-adherence to ART on univariate analysis. On multivariate analysis where gender, baseline WHO clinical stage, baseline CD4 count, baseline BMI and NNRTI were adjusted for the risk of non-adherence was increased by 26% in patients who developed any ART-related toxicity and by 23%, 72%, 43% and 42% among patients who experienced severe anaemia, severe hepatotoxicity, neuropsychiatric toxicity, and skin rash compared with those who did not experience these adverse event. The only adverse event not clearly associated with risk for non-adherence was developing mitochondrial toxicity such as lipoatrophy or peripheral neuropathy.

Table 5.10. A multivariable logistic regression analysis of risk of non-adherence in relation to developing ART-related toxicities among Ghanaian HIV-infected patients.

Toxicity	Non-adherence			
	Unadjusted OR (95% CI)	**p-value**	**Adjusted OR * (95% CI)**	**p-value**
Severe anaemia	1.29 (1.12-1.49)	0.0004	1.23 (1.05-1.44)	0.0096
Severe hepatotoxicity	1.91(1.29-2.83)	0.001	1.72 (1.12-2.64)	0.01
Neuropsychiatric toxicity	1.49 (1.05-2.09)	0.02	1.43 (1.04-1.97)	0.03
Mitochondrial toxicity	0.99(0.73-1.36)	0.96	1.07 (0.76-1.50)	0.71
Skin rash	1.34(1.03-1.74)	0.03	1.42 (1.09-1.85)	0.01
Any toxicity	1.55(1.30-1.86)	<0.0001	1.26 (1.13-1.40)	<0.0001

*after adjusting for gender, baseline WHO clinical stage, baseline CD4 count, baseline BMI and NNRTI. OR is odds ratio.

(7) All-cause discontinuation of NNRTI due to toxicity

There were 55 (3.5%, n=1567; n is number of patients who had at least one follow-up visit after initiating ART) discontinuations of nevirapine compared with 36 (1.6%, n=2234) discontinuations of efavirenz due to drug related toxicity. The median time to discontinuation of nevirapine was 2 months (range, 2 to 56 months) compared with efavirenz of 12 months (range, 2 to 48 months), p=0.0028 (Mann-Whitney's U-test). The unadjusted hazard ratio of discontinuation of nevirapine compared to efavirenz as a result of toxicity was 2.27 (95%CI, 1.55 to 3.46), p<0.0001 as shown in Figure 5.7. In a multivariate Cox proportional model, the risk of discontinuation of nevirapine compared with efavirenz on account of toxicity was 1.89 (1.22-2.92), p=0.004 after adjusting for gender, CD4 count, WHO clinical stage and NRTI backbone. Also, after adjusted analysis females did not have a significantly higher risk of discontinuing NNRTI on account of toxicity compared to males in this model.

(8) All-cause discontinuation of NRTI due to toxicity

There were 62 (3.4%, n=1847) discontinuations of zidovudine due to toxicity compared with 117 (6.0%, n=1954) discontinuations of stavudine due to toxicity. The median time to discontinuation of zidovudine was 2 months (range, 2 to 54 months) compared with stavudine of 18 months (range, 2 to 72 months), p<0.0001 (Mann-Whitney's U-test). As shown in Figure 5.10, the proportionality of baseline hazard assumption was not met for this comparison, so the hazard ratio was not quoted because it is reliable only when this assumption is met.

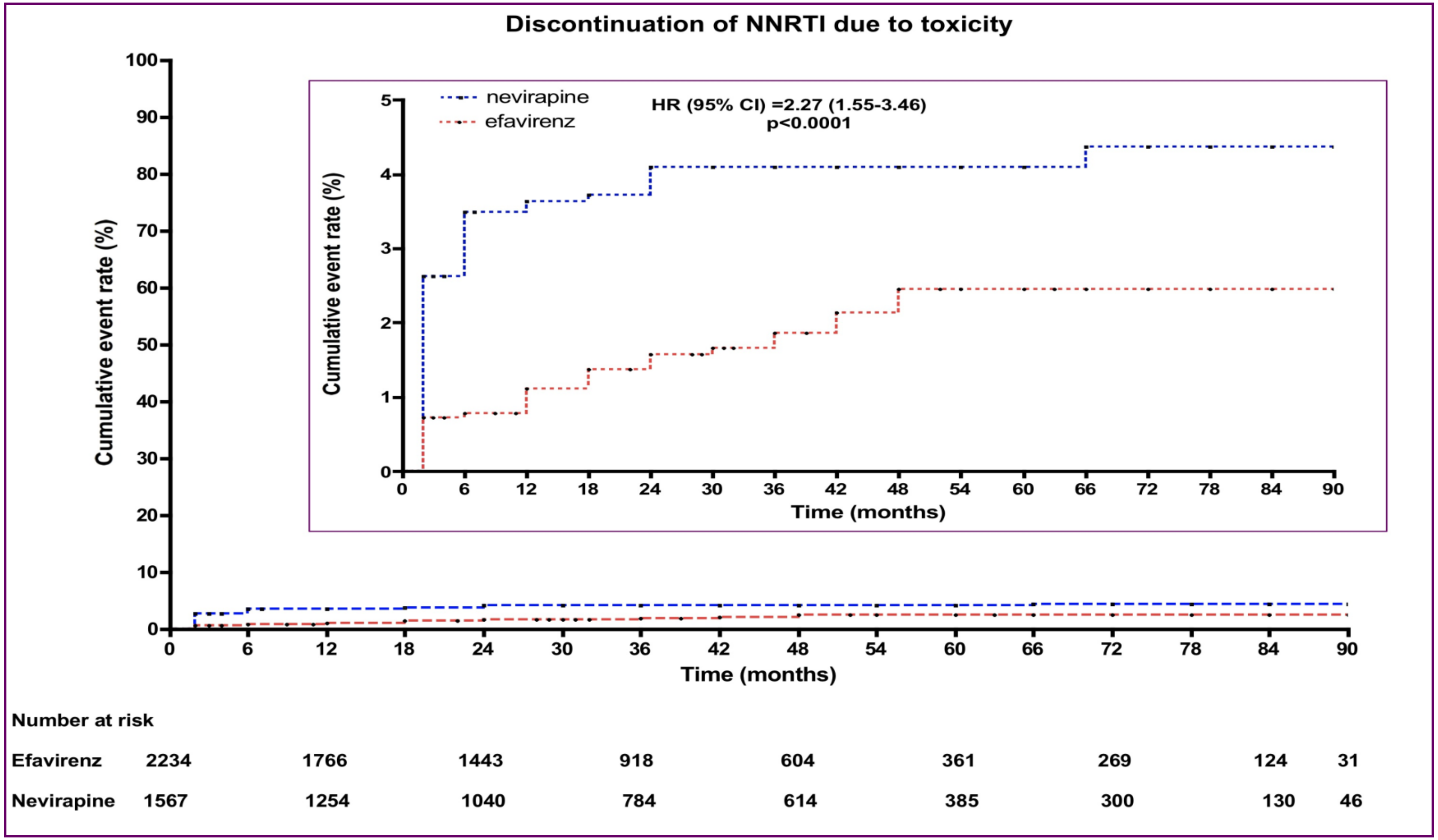


Figure 5.7. Kaplan-Meier estimates of risk of discontinuation of NNRTI due to toxicity.

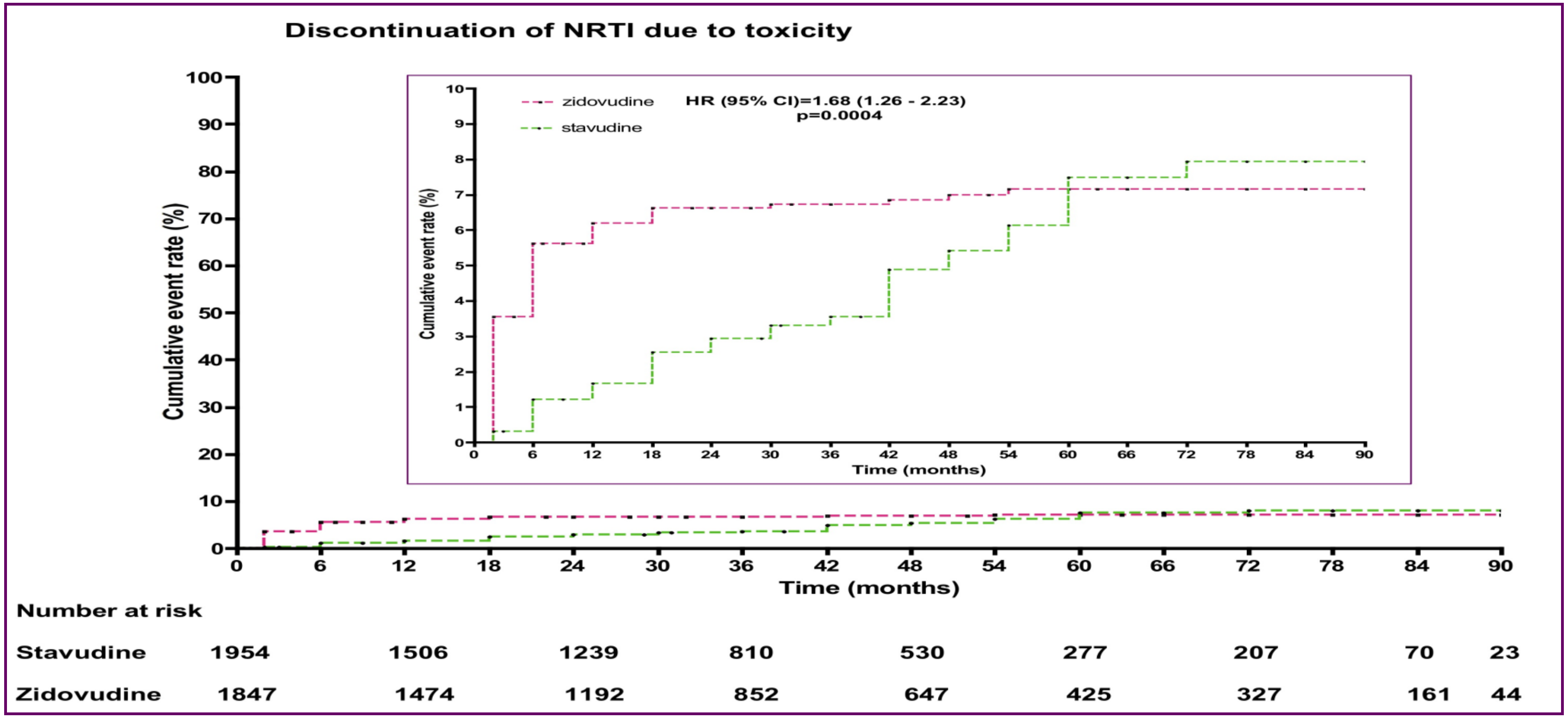


Figure 5.8. Kaplan-Meier estimates of risk of discontinuation of NRTI due to toxicity.

Table 5.11. Frequencies of specific toxicities and treatment switches among Ghanaian cohort on long-term cART.

Toxicity	Number of events	Frequency of events (%)	Number of treatment switched due to toxicity	Frequency of treatment switch due to toxicity (%)
Anaemia	675	41.5	62	9.2
Skin rash	299	18.4	44	14.7
Neuropsychiatric toxicity	235	14.4	39	16.6
Peripheral neuropathy	181	11.2	83	45.9
Severe hepatotoxicity	160	9.8	8	5.0
Lipoatrophy	40	2.5	34	85.0
Ptylism	14	0.9	0	0.0
Gastrointestinal disorders	12	0.7	0	0.0
Lactic acidosis	4	0.2	NA	NA
Myalgia	3	0.2	0	0.0
Hyperpigmentation	3	0.2	0	0.0
Pancreatitis	1	0.1	1	100.0
Total events	1,627	100.0%	271	16.7%

Frequency of events = number of specific events / total number of events

Frequency of treatment switch due to toxicity= number of treatment switched due to specific toxicity/ number of specific events

Discussion

HIV is now viewed as a chronic disease requiring life-long therapy with potent combinations of antiretroviral medications. The prevention and management of antiretroviral therapy-related toxicity has emerged as one of the major issues for HIV/AIDS management[485, 486]. Treatment-associated toxicities has been identified as one of the major reasons for treatment discontinuation[149, 482,483] and an important predictor of poor adherence for which reason long-term toxicity data is required, particularly in resource limited settings where treatment options are limited. The present data shows that there were 1,627 documented episodes of ART-related toxicities among 4,039 Ghanaian HIV-infected patients who started first line ART with an overall event rate of 14.5 events/100 person-years (95% CI of 13.8 to 15.2/100 person-years). As summarised in Table 5.11, severe anaemia, skin rash and neuropsychiatric toxicity were the commonest reported adverse events with an overall treatment modification frequency of 16.7%, however treatment limiting events necessitating a switch varied between 0% to 100% depending on the specific toxicity.

Severe anaemia was the commonest toxicity recorded among this Ghanaian cohort occurring at a frequency of 41.5% of all ART-related toxicities and at a median time of 2 months with a range of 2 to 78 months. In a multivariate analysis, the risk factors identified for the development of severe anaemia among our cohort were severe anaemia at baseline, a low BMI below 18.5kg/m^2, AIDS diagnosis at baseline and a CD4 count below 200 cells/mm^3. Of interest, neither the use of zidovudine vs stavudine nor efavirenz vs nevirapine was clearly associated with the risk of developing severe anaemia in this analysis. This would indicate that the occurrence of anaemia as a toxicity is determined by baseline characteristics of the patients with those with advanced disease more likely to develop this event than those with earlier disease. Evidence from the DART study shows that in resource-limited settings, the risk factors for developing severe anaemia after initiating ART are female sex, baseline anaemia, low CD4 counts and low BMI with our data confirming 3 out of these 4 reported risk factors[487]. Furthermore, these same risk factors were identified to be associated with severe anaemia even before initiation of ART in another study[488]. Consequently attributing the cause of severe anaemia as toxicity to ART within the first few months of initiating therapy particularly among patients with advanced disease may be problematic hence the analysis thus presented should be viewed as one of associations not causative factors of anaemia. Another reason for this supposition from our data is the median time to occurrence of severe anaemia was within 2 months of initiating therapy. Clearly, these patients may not have had enough time on treatment to recover from the profound effects of HIV infection at this time point. There is the potential for on-going symptoms of HIV to be mis-diagnosed as toxicities necessitating premature treatment changes, hence stricter definitions of clinical toxicities may be useful to assist in diagnostic overlaps. The proposed mechanisms of anaemia in HIV disease involves an interruption of haematopoiesis through disruption of several cytokine mechanisms by HIV infection of bone marrow stromal cells and macrophages[489]. Use of zidovudine may be associated with severe anaemia however two separate models that took into account whether anaemia was present or absent at time of initiating cART did not reveal an independent association between zidovudine and risk of severe anaemia. Our data therefore suggests if zidovudine is carefully selected as part of initial ART regimens, the risk of developing severe anaemia on ART were similar among zidovudine- and stavudine-recipients and that this risk is defined by patients characteristics rather than the specific components of the cART. Thus said among a subset of patients who started with no baseline anaemia, the use of zidovudine was more frequently associated with profound and life-threatening decreases in haemoglobin concentrations.

Skin rash was the most commonly reported NNRTI-related adverse event with an incidence rate (95% CI) of 2.66 (2.37 – 2.98) events/100 person-years of follow up with a median time to occurrence of 2 months. The overall frequency of NNRTI-related skin rash in our cohort was 7.0% with a higher frequency of 10.2% among patients on nevirapine than 5.6% among efavirenz recipients. The reported frequencies of nevirapine-related rash range between 4% -38% [4, 21, 22] of patients while that of efavirenz is between 4.6%-20% among different ethnicities[4, 22, 23]. Thus the incidence of NNRTI-related skin rash within the present cohort is at the lower end of the spectrum. Severe to life-threatening skin rash were reported at a low frequency of 0.7% of nevirapine recipients and 0.2% of efavirenz recipients with 2 fatalities associated with nevirapine related skin rash, although this may be an under-estimation as potentially those who develop life-threatening and fatal skin rash may not have reported back to the clinic. Nevirapine was administered at a dose of 200mg once daily for the first two weeks as a lead-in dose followed by an escalation to 200mg twice daily according to locally recommended guidelines. When administered in this fashion, the risk for nevirapine related skin rash has been found to be reduced by 50%[198,199].The risk factors identified in association with skin rash on adjusted analysis included nevirapine use, female gender and low baseline CD4 T-cell counts while higher WHO clinical stage, age lower than 40 years, lower BMI and hepatitis B sero-positivity were found to be of significance in unadjusted analysis (Table 5.4).

A cell-mediated hypersensitivity reaction has been postulated as one of the possible mechanisms for the development of NNRTI related skin rashes[21, 22, 190, 191]. Indeed in our cohort, a lower CD4 T-cell count at the time of initiating therapy followed by a more profound recovery in CD4 counts measured within 6 and 12 months were factors identified in association with NNRTI-related skin rash. Thus our data support a mechanism akin to an immune reconstitution inflammatory syndrome where rapid restoration of CD4 T-cells elicit inflammatory responses to as yet to-be identified antigenic epitopes of which the NNRTI itself could be one among several other potential candidates. There is however a contrast between our data and that reported among Thai patients[22] (n=210), in whom NNRTI skin rash was commoner among patients who had higher CD4 counts at baseline, had earlier clinical stages of HIV disease and had higher BMI. These differences could be due to differences in ethnicity and possibly genetic predispositions. Female gender was significantly associated with NNRTI-related rash as has been reported elsewhere[490-492] and it is thought to be related to hormonal differences, gender related differences in cytochrome P450 metabolism or body size. However in many cohorts like ours, females are more adherent, less likely to withdraw from ART care and therefore more likely to report any adverse events.

Neuropsychiatric toxicity due to NNRTI use has predominantly been reported among efavirenz recipients with a frequency ranging from 25% to 70% in various studies[23 ,201-204]. In this cohort, the reported frequency of neuropsychiatric toxicity on efavirenz was 7.6% while that on nevirapine was 2.4% with an admittedly low overall frequency of 5.5% compared with other reports. It is well-known that most neuropsychiatric toxicities are mild to moderate and resolve after the first few weeks of therapy[23, 201 - 204, 206] and therefore may not have been reported or documented in patients' records. As expected, the median time to first episode of neuropsychiatric toxicity was within the first 2 months upon initiating therapy with most events occurring within the first year but as noted in Figure 5.4, even after the first year of therapy, some patients experienced toxicity as indicated by other[205, 206, 208] authors. Whether the reported toxicities after more than 1 year of therapy represents exacerbations of chronic undocumented toxicity or new events occurring after continual use of efavirenz cannot be confidently ascertained due to the retrospective nature of this study. Again, the severity of neuropsychiatric toxicity and its impact on quality of life was not assessed with objective instruments such the Profile of Mood State (POMS-A) and Medical Outcomes Study-HIV (MOS-HIV)[493] questionnaires and requires more studies to evaluate them further in the Ghanaian context.

Most studies reporting efavirenz associated neuropsychiatric toxicity have been based on short-term follow-up of a few weeks but a cross-sectional study by Fumaz et al. among patients (n=60) who had been on efavirenz for a mean time of 91.1 ± 39.5 weeks found that mild and clinically tolerable neuropsychiatric disorders persisted[255]. Our data shows that even up to 90 months, some patients reported experiencing efavirenz related neurotoxicity for which therapy changes were not performed, indicating that they may have been tolerable for the patients. Among our cohort, insomnia, headache, dizziness, abnormal dreams and drowsiness were reported at overall frequencies of 50.0%, 7.5%, 6.7%, 5.6% and 5.2% respectively with higher incidences among efavirenz recipients compared with nevirapine recipients. Rarely reported events such as seizures, cerebellar disorders and suicidal ideations were reportedly attributed to efavirenz recipients by treating clinicians and these resolved after discontinuation of the implicated efavirenz. Cranial computed tomography scans were performed in the 3 reported cases of seizures and 2 cases of cerebellar ataxia but were found normal. However it is well known that seizures and cerebellar disorders could be due to non-space occupying lesions such as HIV infection itself. But the resolution of these events upon discontinuation of efavirenz suggests that efavirenz may have contributed to its occurrence.

The mechanisms for CNS toxicity caused by efavirenz remains to be elucidated. There are suggestions that exposure to supra-therapeutic levels due to slower metabolism of efavirenz from the highly prevalent CYP2B6 516 G>T polymorphisms among African Americans for instance could explain why neuropsychiatric disturbances are commoner among them than in European American or Hispanic patients[209]. Others have postulated that the neuropathic effects of HIV itself, the effect of efavirenz on cytokine homeostasis, previous predisposition towards neuropsychiatric disturbances and sleep disturbances may all contribute to central nervous system (CNS) toxicity[205, 206]. In this cohort, the only risk factors for neuropsychiatric toxicity were the use of efavirenz, 229% higher risk compared with nevirapine, and a low body mass index below 16kg/m^2 with a 44% higher risk in adjusted multivariate analysis. This suggests that patients of low body weight may be exposed to higher concentrations of a fixed dosage of 600mg daily and thus predispose them to neuropsychiatric toxicity. In our cohort 17% of patients with documented CNS toxicity discontinued efavirenz due to neurotoxicity which is slightly on the higher side of the reported 4% to 10% of patients who discontinued efavirenz due to CNS or neuropsychiatric toxicity[23, 201 - 206]. This higher proportion of treatment-limiting CNS toxicity on efavirenz should viewed within a context of the possibility of under-reported events. However, the possibility also exists for higher therapeutic exposure to efavirenz because of the ethnicity defined high prevalence of mutant polymorphisms in the hepatic enzymes responsible for the metabolism of efavirenz as has been explored in chapter 8. The lower incidence of these neuropsychiatric events among patients on nevirapine compared with efavirenz and the fact that all patients who switched from efavirenz to nevirapine did not experience any further episodes supports the recommendations for efavirenz to be switched to nevirapine in the few patients in whom CNS toxicity persists, is severe or have increased predisposition for developing them.

Hepatotoxicity is one of the well-recognised components of the broad spectrum of antiretroviral therapy toxicity and elevations in serum hepatic enzymes have been described in association with all the major classes of antiretroviral drugs[28, 211, 494-501]. The frequency of severe hepatotoxicity among our cohort was 3.9%. There was no difference in the frequency of severe hepatotoxicity among efavirenz recipients-3.7% compared to 3.8% among nevirapine recipients, which are within the lower limits of reported frequencies of 1.1-8% and 1.4-17% in various studies[10, 24-28]. Thus the predisposition to this toxicity was comparable between the two NNRTIs among this Ghanaian cohort although nevirapine-associated hepatotoxicity occurred at an earlier median time of 6 months compared with 12 months in efavirenz-related hepatotoxicity, p=0.03.

Among the several mechanisms proposed for the occurrence severe hepatotoxicity are: hypersensitivity reaction in association with non-nucleoside reverse transcriptase inhibitors (NNRTIs), mitochondrial toxicity in association with several nucleoside reverse transcriptase inhibitors (NRTIs) and immune reconstitution inflammatory syndrome in association with chronic viral hepatitis[28, 211, 494-501]. Of these proposed mechanisms our data supports the role of chronic viral hepatitis in causing severe hepatotoxicity on antiretroviral therapy in the Ghanaian population. Patients with hepatitis B co-infection had a 99% (95% CI 16% to 240%, p=0.01) higher risk of experiencing severe hepatotoxicity compared with sero-negative patients. It should be emphasized that hepatitis B screening data was not available for all patients and thus these results should be viewed as a sub-analysis of the whole cohort and thus incidence rates should be interpreted cautiously. HCV serology was also not available for all patients. Although the incidence of severe hepatotoxicity was high among HBV sero-positive patients there were not differences in the risk among patients who were either on nevirapine or efavirenz. Among Thais, the risk of severe hepatotoxicity was highest among HBV sero-positive patients on nevirapine[362] but this was not the case in our cohort. Over the long-term, these severe hepatoxicity were not associated with adverse outcomes such as death, in fact most transaminitis resolved with continual follow up with only 5.6% of events leading to NNRTI switch.

The two most commonly reported mitochondrial toxities in this cohort were peripheral neuropathy and lipoatrophy with reported overall frequencies of 6.7% (95% CI of 5.6% to 7.8%) and 2.9% (95% CI of 1.9% - 3.8%) respectively. Among patients who were on stavudine the principal cause of these two toxicities, the frequencies were 11.3% (95%CI of 9.3% to 13.2%) and 6.5% (95% CI of 4.3% to 8.8%) over the median follow-up duration of 30 months (range 0-90 months). Among African cohorts the reported frequency of peripheral neuropathy and lipoatrophy was 20.7% and 2.2% (n=1,286) among Kenyans after 2 years of follow up[433]. However in a Cambodian cohort where patients were intensively screened for stavudine related toxicity, the frequency of peripheral neuropathy and lipoatrophy among stavudine recipients were 19.0% and 72.4% respectively after follow up of 60 months[502]. Thus the low frequency again could to due to under-reporting or could be due to the phasing out of stavudine usage from our national programme since the early 2009 upon recommendations of the WHO. Among the various ART medications the one most discontinued among Ghanaians is stavudine.

The median time to the onset of peripheral neuropathy was 6 months and that of lipoatrophy was 42 months which generally supports the idea that these two principally stavudine-mediated toxicities are slow in developing. Thus in settings such as ours where options for changing are limited, a strategic phasing out of stavudine as has already been started may be implemented among carefully selected patients based on risk factors specific to this population. Among the risk factors identified in association with stavudine related toxicity were female gender, age>40 years and low haemoglobin concentrations as has also been reported among Cambodians[502]. Anaemia at baseline though is the primary reason for selecting stavudine for these patients and therefore it is difficult to completely extricate the cause- and effect- association here. Four fatal cases of suspected lactic acidosis was reported in our cohort. Indeed, it is possible that the incidence of lactic acidosis on stavudine may be higher than reported as can be observed by the high numbers of patients on stavudine who died or were lost to follow up within the first 6 months of initiating therapy (chapter 4). The true incidence of lactic acidosis or hyperlacticacidaemia is difficult to estimate due to the lack of facilities for its routine measurement.

It has been clearly demonstrated in that the occurrence of adverse events on cART is linked with the risk of poor adherence to therapy[503-505]. Our data corroborates these observations by showing that the occurrence of any toxicity on cART was associated with a 26% higher odds of sub-optimal adherence to therapy. This figure varied with different toxicities as shown in Table 5.10 with varied treatment discontinuation responses by clinicians (Table 5.11). For instance, it was observed that experiencing severe hepatotoxicity was associated with a higher odds of 72%, p=0.001 for sub-optimal adherence however clinicians were willing to change therapy in only 5.6% of patients with this event. These low treatment discontinuations rates due to toxicity may reflect clinician preferences and limited options for changing therapy. These observations draw links between toxicity and adherence which consequently affects the long-term durability of cART.

Certainly the durability of any cART regimen depends amongst other factors on its tolerability and its efficacy and this analysis in agreement with other published data suggests a significantly higher rate and earlier onset of discontinuation of nevirapine compared with efavirenz due to toxicity. Specifically, compared with efavirenz-based cART, patients initiating nevirapine-based cART were at a 89% higher risk of discontinuation due to treatment-limiting toxicity (p<0.0001). The common reasons for discontinuation of nevirapine as highlighted are hypersensitivity reactions such as skin-rash and severe hepatotoxicity while efavirenz was commonly discontinued on account of neuropsychiatric toxicity. This is a significant observation highlighting a greater risk of treatment-limiting toxicity on nevirapine compared with efavirenz and has implications for developing strategies for identifying patients at higher risk for nevirapine toxicity and thus initiating therapy with efavirenz instead. These issues are dealt with in further details in chapter 6.

A number of limitations are worth mentioning in interpreting the data presented. This is a retrospective analysis, using data from a treatment programme setting. Therefore the reported rates of events are likely to under-estimate the real rates since some patients with severe toxicity may have been lost to attrition from the programme. For instance, the high withdrawal rates among males (chapter 4) means that any associations of between toxicity and gender should be interpreted with caution. There is also the possibility that events that were of mild nature may not have documented. However, patients were systematically evaluated at each clinical visit by clinicians, with standard clinical assessment, patient management and reporting. Still, it remains that the unavailability of technical investigations to more rigourously diagnose the different toxicites could have led to misclassifications. For example, there were no facilities on site to measure lactic acid concentrations in the sera of patients to better diagnose hyperlacticacidaemia nor was a dual energy X-ray absorptiometry (DEXA) scan available for accurate characterisation of lipoatrophy which had a low documented incidence overall. Thus said, the reported data demonstrate associations, and not causation.

In summary, although toxicities due to specific agents were commonly reported, their incidence rates were generally lower than reported from other cohorts. Treatment discontinuations were few and patients generally did not experience worsening of events when maintained on their medications. Patients were more likely to discontinue nevirapine than efavirenz on account of documented toxicity. Toxicity was associated strongly with the risk for sub-optimal adherence. We next explore the long-term effectiveness of efavirenz-based cART with nevirapine based cART (chapter 6) and in chapter 8 associations between random plasma concentrations of efavirenz and risk for CNS toxicity and immunological failure is presented.

CHAPTER SIX

6.0 Comparison of long-term clinical and immunological responses to efavirenz- or nevirapine-based first line antiretroviral therapy among Ghanaians

6.1 Introduction

The World Health Organisation guidelines recommend that for resource-constrained settings, combination antiretroviral therapy (cART) should be initiated with a non-nucleoside reverse transcriptase inhibitor (NNRTI), specifically either efavirenz or nevirapine with two nucleoside reverse transcriptase inhibitors (NRTIs) as backbone[1,506]. In spite of the widespread use of these NNRTIs, evidence of their effectiveness has been conflicting. A systematic review[31] of seven randomised controlled trials found clinical equivalence of efavirenz and nevirapine[4, 507-512]. However clinical, immunological, virological and toxicity data from observational studies[5-20] conducted in low-, middle-, and high-income countries are quite disparate from those of experimental studies. For instance, the 2NN study (a large, placebo, open-label randomised controlled study) showed non-inferiority of nevirapine to efavirenz[4]. However, in one large observational well-attended, private-sector cohort study, among 2,817 HIV-infected South Africans who initiated either efavirenz-based (64.7%) or nevirapine-based cART (35.5%), efavirenz was associated with superior clinical and virologic outcomes compared with nevirapine over a median follow-up time of 2.2 years (range of 1 month to 3 years)[8].

On the basis of its more favourable toxicity profile and efficacy data, efavirenz is indeed preferred to nevirapine for initial therapy in resource-endowed countries[111, 190] . But a WHO-led survey found that almost 67% of countries in Sub-Saharan Africa recommend nevirapine-based regimens for first-line therapy because of lower cost and its availability in generic fixed dose combination regimens[513, 514]. But the use of nevirapine is limited by its toxicity profile and also by its adverse interactions with anti-tuberculous medications given to treat the vast majority of patients with TB-HIV co-infection who often would need to initiate therapy for both infections concurrently[515, 516].

In a meta-analysis comparing these two NNRTI's the authors submitted that the differences in efficacy between efavirenz- and nevirapine-based cART may be subtle and that more studies are required to determine whether differences emerge over the long-term use[31]. Furthermore, in spite of the widespread use of these two NNRTI in Sub-Saharan Africa in routine clinical care, their long-term clinical and immunological outcomes have seldom been evaluated in these programmatic settings. The main objective of this chapter is therefore to compare the long term clinical and immunological outcomes of efavirenz-based cART with nevirapine-based cART among ART naïve HIV infected Ghanaians initiating therapy with two thymidine NRTI backbone of either zidovudine plus lamivudine or stavudine plus lamivudine. In chapter 4 of this dissertation, it was shown that the hazards of disease progression, loss-to-follow up and death were not significantly different among patients initiating either EFV or NVP but in chapter 5 it emerged that the risk for discontinuation of NNRTI on account of toxicity was significantly higher for nevirapine than efavirenz. Therefore in the present analysis, the primary outcome measure of treatment failure was a composite of deaths, clinical progression and all-cause treatment discontinuations using an intention-to-treat analysis. Secondary outcome analyses were performed to compare CD4 T-cell count changes, changes in body mass index and the impact of the NRTI backbone on the primary and secondary outcome measures of these two NNRTIs.

6.2 Methods

Please refer to chapter 2.6

6.3 Results

6.3.1 Patient characteristics

Included in this analysis are a total of 3,990 patients who started either an efavirenz-based (n=2,369; 59.4%) or nevirapine-based (n=1,621; 40.6%) cART between January 2004 and December 2010. Forty-nine (49) patients were excluded because they started on protease inhibitor-based cART (n=40) and 9 started with NRTI backbones other than zidovudine plus lamivudine or stavudine plus lamivudine.

As shown in Table 6.1, there is a female predominance in a ratio of 2:1 with a lower proportion of patients starting nevirapine-based cART being males (15.2%) compared with those starting efavirenz-based cART (43.4%). Patients starting efavirenz-based regimen were older [median age of 40 years, IQR of 35 to 47] and heavier [median weight of 51kg, IQR of 45 to 60 kg] than those starting nevirapine-based regimen with median age of 35 years (IQR, 30-42) and weight of 50kg (IQR, 44-59) respectively. There were however no differences in the proportion with prior diagnosis of AIDS in the two groups before initiation of therapy. The baseline median (IQR) CD4 count among patients initiating efavirenz-based cART was significantly lower compared with those initiating nevirapine-based cART; 127 (45-213 cells/mm^3) vs 140 (56-220 cells/mm^3), p=0.004. Although patients starting efavirenz-based cART had significantly higher baseline serum concentrations of aspartate transaminase, alanine transaminase and creatinine than those starting nevirapine-based cART, these differences were clinically deemed not significant and were thought to reflect the male gender bias among those initiating efavirenz. Also there were no differences in the serological status of hepatitis B co-infected patients. Of patients who commenced efavirenz-based cART, 45.7% commenced with an NRTI backbone of zidovudine plus lamivudine compared with 50.4% starting on nevirapine-based cART while 54.3% and 49.6% starting stavudine plus lamivudine respectively.

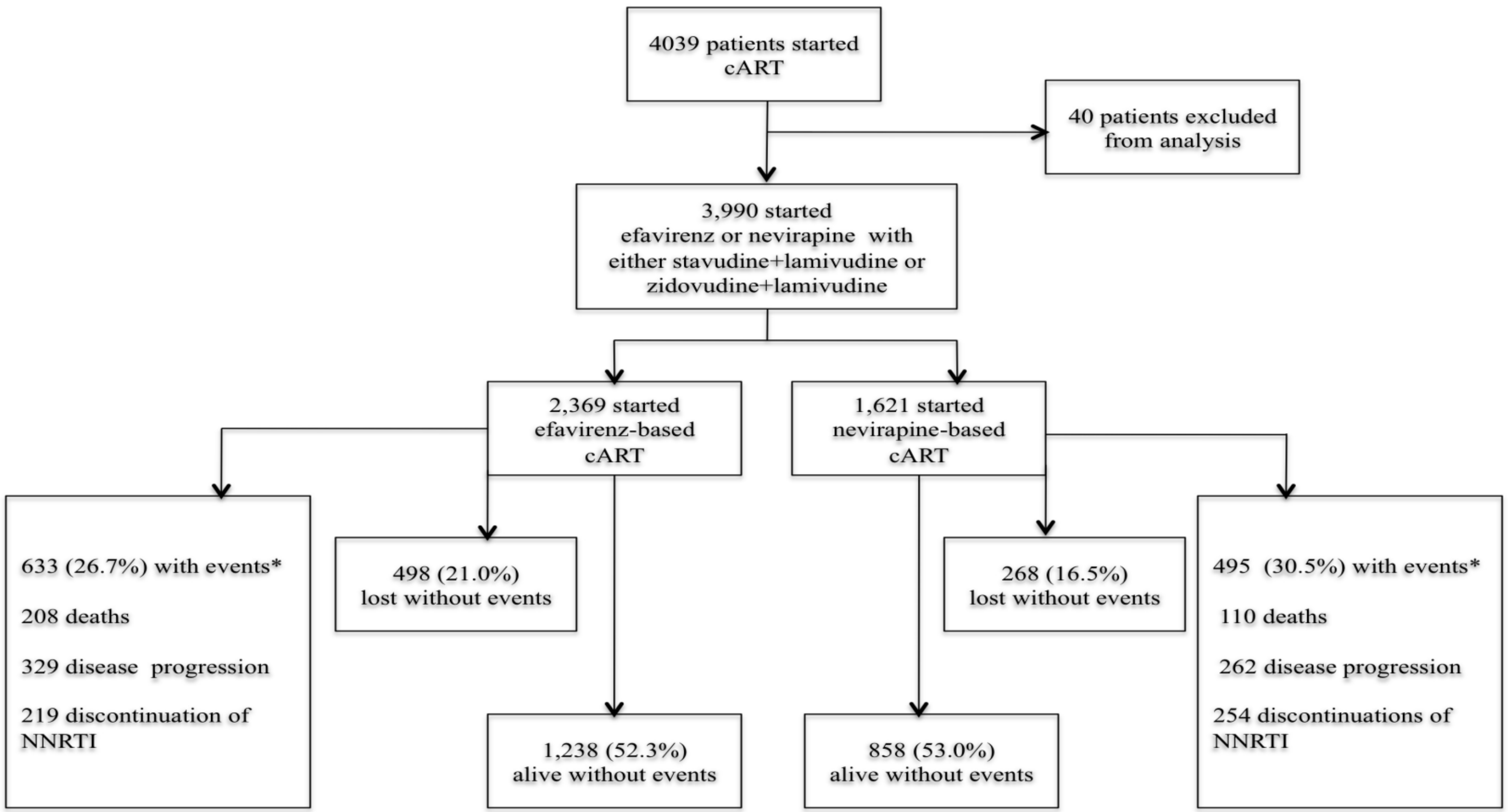
Table 6.1 Characteristics of patients at time of starting combination anti-retroviral therapy.

Characteristic	Efavirenz, n=2,369		Nevirapine, n=1,621		p-value
	n	%	n	%	χ^2
Gender					
Male	1027	43.4	246	15.2	<0.0001
Female	1342	56.6	1375	84.8	
Prior AIDS					
Yes	1677	70.8	1105	68.2	0.13
No	423	17.9	329	20.3	
Unknown	269	11.3	187	11.5	
Hepatitis B status					
Negative	1061	44.8	785	48.4	0.08
Positive	180	7.6	116	7.2	
Unknown	1128	47.6	720	44.4	
NRTI backbone					
AZT and 3TC	1083	45.7	817	50.4	0.0036
D4T and 3TC	1286	54.3	804	49.6	
Calender year					
2004	402	17.0	349	21.5	<0.0001
2005	368	15.5	324	20.0	
2006	429	18.1	384	23.7	
2007	439	18.5	214	13.2	
2008	425	18.0	159	9.8	
2009	175	7.4	96	5.9	
2010	131	5.5	95	5.9	

	Median	IQR	Median	IQR	U-test
Age (years)	40	35-47	35	30-41	<0.0001
Body weight (kg)	51	45-60	50	44-59	0.05
BMI (kg/m^2)	19.6	17.4-22.3	19.9	17.6-22.9	0.002
CD4 count (cells/μl)	127	45-213	140	56-220	0.004
Haemoglobin conc. (g/dl)	10.2	8.8-11.5	10.2	8.8-11.5	0.85
Serum AST concentration (IU/l)	42	30-60	38	28-52	<0.0001
Serum ALT concentration (IU/l)	31	21-46	26	19-37	<0.0001
Serum creatinine conc. (μmol/l)	88.4	70.7-110.5	79.6	67.0-98.4	<0.0001

6.3.2. Composite end-point analyses

At closure of data for analysis, 2,096 (52.5%) patients were alive and still under follow up with none of the events of the composite outcome measures of treatment failure- 1,238 (52.3%) were still on efavirenz-based cART and 858 (53.0%) were still on nevirapine-based cART without any event as shown in Figure 6.1. Seven hundred and sixty-six (19.2%) patients were lost to follow-up without experiencing any of the three events used as a composite end-point of therapy failure, 498 (21.0%) were on efavirenz and 268 (16.5%) were on nevirapine. One thousand one hundred and twenty-eight (1,128) representing 28.3% of patients initiating either one of these NNRTIs had at least one event in the composite end-point- 633 (26.7%) for efavirenz versus 495 (30.5%) for nevirapine. Among efavirenz recipients with therapy failure, 4 (0.6%) experienced all three events, 117 (18.5%) experienced 2 out of 3 events and 512 (80.9%) experienced 1 out the 3 co-primary events of the composite end-point. Eleven patients (11, representing 2.2%) experienced all three events under follow up, 107 (21.6%) experienced 2 out of 3 events and 377 (76.2%) experienced one out of 3 co-primary events among nevirapine recipients. Although patients could have more than one of these events, only the earliest event was modelled in the Cox analysis.



* A patient could have more than one of the composite events

Figure 6.1. Characterisation of study participants by composite treatment outcome measure

Figure 6.2 shows the Kaplan-Meier analysis for risk of composite events between the two NNRTIs with a hazard ratio and log-rank p-value of 0.90 (95%CI of 0.79 to 1.01) and 0.07 respectively among patients on efavirenz compared with nevirapine. In primary analyses, the risk of therapy failure among patients on nevirapine compared with efavirenz were 1.20 (0.97 to 1.49), p=0.10 in adjusted analysis using the Cox proportional hazards regression and 1.09 (0.79 to 1.49), p=0.61 after adjustment in the logistic regression model. Given the inequality of loss-to-follow up among the two treatment groups, sensitivity analyses compared the risk of therapy failure to account for confounding due to missing patients where loss to follow up was treated as failure. Similarly, in these sensitivity analyses the adjusted hazards ratio and odds ratio of therapy failure among patients on nevirapine compared with efavirenz were 0.97 (0.82-1.15), p=0.73 and 0.98 (0.77-1.24), p=0.83 respectively.

The main determinants of the composite outcome in both primary and sensitivity analyses with both models were initiating therapy below 40 years old, CD4 T cell counts below 200 cells/mm^3, BMI <16kg/m^2, AIDS diagnosis at baseline and poor adherence to therapy as shown in Tables 6.2 and 6.3. The combination of stavudine plus lamivudine was associated with an adverse composite outcome only in sensitivity analysis in both models. Because the composite end-point was an admixture of several treatment outcome measures, there was the need to explore the risk factors for each of these components to examine for differences if any between efavirenz- and nevirapine-based cART. The risk factors for death, loss to follow up, disease progression on cART with respect to NNRTI have already been presented in Chapter four and the risk for discontinuation on NNRTI due to toxicity in Chapter 5. The analyses presented below look at the risk for NNRTI discontinuations.

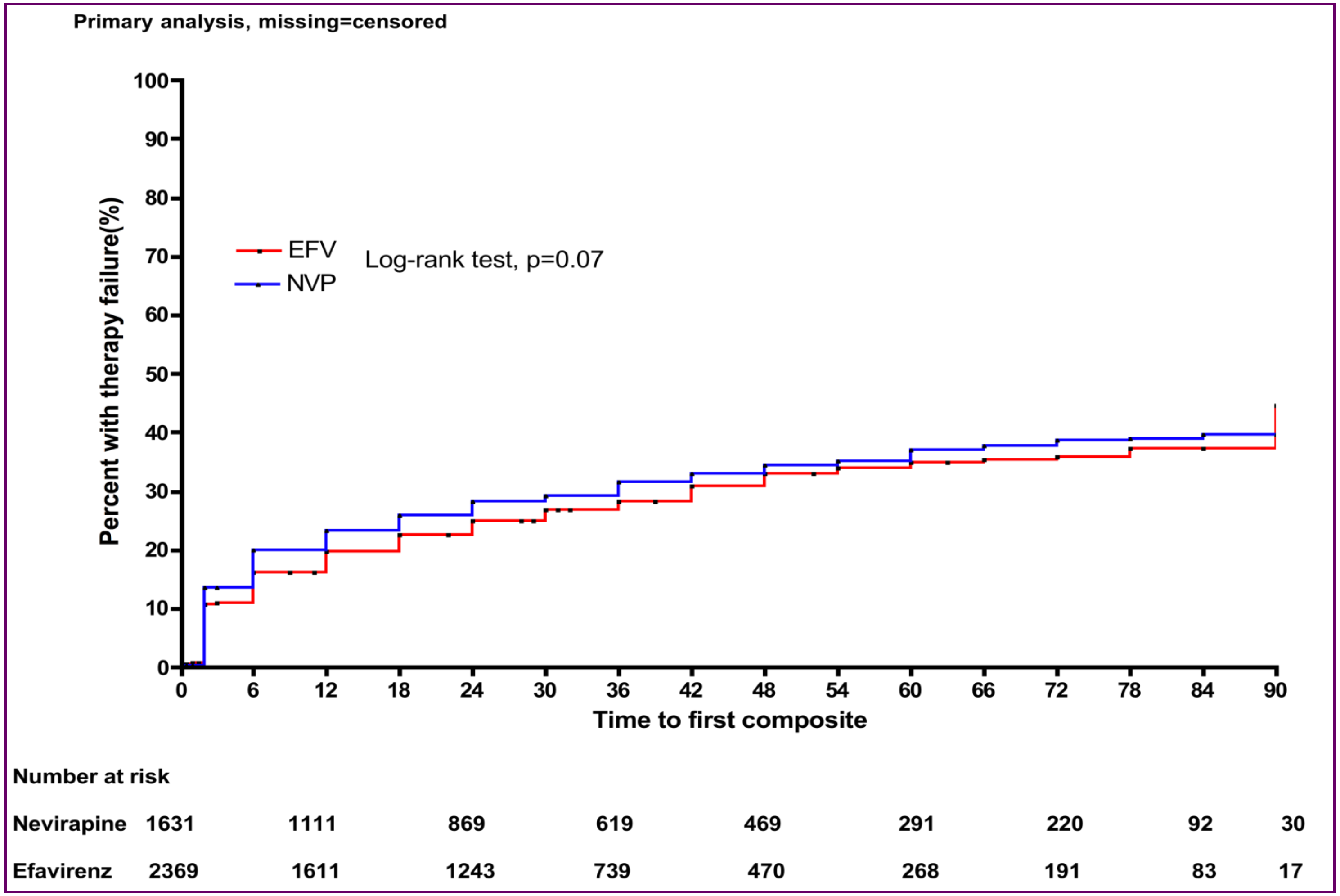


Figure 6.2. Kaplan-Meier analysis showing risk of treatment failure defined as a composite of deaths, disease progression and NNRTI discontinuations.

Table 6.2. Univariate and multivariate Cox proportional hazards regression analysis of factors associated with composite failure on either efavirenz- or nevirapine-based first line cART (showing significant factors only**).**

	Missing = Censored (Primary analysis)				Missing = failure (sensitivity analysis)			
Variable	**Unadjusted HR**	**p-value**	**Adjusted HR**	**p-value**	**Unadjusted HR**	**p-value**	**Adjusted HR**	**p-value**
NNRTI								
Nevirapine	1.07(0.95-1.21)	0.24	1.20(0.97-1.49)	0.10	0.94 (0.86-1.03)	0.17	0.97 (0.82-1.15)	0.73
Efavirenz	1.00		1.00		1.00		1.00	
NRTI backbone*								
D4T plus 3TC	1.25(1.12-1.41)	0.0002	1.10(0.96-1.25)	0.16	1.30 (1.18-1.42)	0.0000	1.32 (1.05-1.65)	0.02
AZT plus 3TC	1.00		1.00		1.00			
Age								
<40 years	1.19(1.05-1.34)	0.004	1.31(1.12-1.54)	0.0006	1.11 (1.01-1.22)	0.02	1.17 (1.04-1.31)	0.009
≥ 40 years	1.00		1.00		1.00		1.00	
Baseline CD4 strata								
<200 cells/mm^3	1.63(1.42-1.88)	0.0000	1.47(1.27-1.69)	0.0000	1.62 (1.45-1.80)	0.0000	1.42 (1.28-1.59)	0.0000
≥200 cells/mm^3	1.00		1.00		1.00		1.00	
Baseline BMI								
<16 kg/m^2	1.90(1.63-2.22)	0.0000	1.64(1.40-1.92)	0.0000	1.86 (1.66-2.10)	0.0000	1.63 (1.44-1.84)	0.0000
≥16 kg/m^2	1.00		1.00		1.00		1.00	
WHO clinical stage								
3 or 4	1.59(1.35-1.88)	0.0000	1.38(1.16-1.63)	0.0002	1.60 (1.41-1.83)	0.0000	1.38 (1.21-1.58)	0.0000
1 or 2	1.00				1.00		1.00	
Adherence								
Poor	1.30 (1.16-1.47)	0.0000	1.30(1.16-1.47)	0.0000	1.29 (1.18-1.42)	0.0000	1.26 (1.15-1.38)	0.0000
Excellent	1.00		1.00		1.00		1.00	

Table 6.3. Univariate and multivariate logistic regression analysis of factors associated with composite failure on either efavirenz- or nevirapine-based first line cART (showing significant factors only)**.**

	Missing = Censored (Primary analysis)				Missing = failure (Secondary analysis)			
Variable	**Unadjusted OR**	**p-value**	**Adjusted OR**	**p-value**	**Unadjusted OR**	**p-value**	**Adjusted OR**	**p-value**
NNRTI								
Nevirapine	1.20 (1.04-1.38)	0.01	1.09 (0.79-1.49)	0.61	0.98 (0.87-1.12)	0.79	0.98 (0.77-1.24)	0.83
Efavirenz	1.00		1.00		1.00		1.00	
NRTI backbone*								
D4T plus 3TC	1.18 (1.03-1.36)	0.02	1.06 (0.91-1.23)	0.48	1.35 (1.19-1.53)	0.0000	1.20 (1.04-1.38)	0.01
AZT plus 3TC	1.00		1.00		1.00		1.00	
Age								
<40 years	1.25 (1.08-1.43)	0.002	1.40 (1.17-1.69)	0.0003	1.18 (1.04-1.34)	0.0083	1.31 (1.10-1.55)	0.002
≥ 40 years	1.00		1.00		1.00		1.00	
Baseline CD4 strata								
<200 cells/mm^3	1.72 (1.46-2.01)	0.0000	1.57 (1.33-1.85)	0.0000	2.02 (1.76-2.33)	0.0000	1.72 (1.48-1.99)	0.0000
≥200 cells/mm^3	1.00		1.00		1.00		1.00	
Baseline BMI								
<16 kg/m^2	1.70 (1.43-2.06)	0.0000	1.48 (1.22-1.81)	0.0001	2.18 (1.80-2.63)	0.0000	1.86 (1.52-2.27)	0.0000
≥16 kg/m^2	1.00		1.00		1.00		1.00	
WHO clinical stage								
3 or 4	1.56 (1.29-1.89)	0.0000	1.37 (1.12-1.66)	0.002	1.83 (1.55-2.16)	0.0000	1.52 (1.28-1.81)	0.0000
1 or 2	1.00				1.00		1.00	
Adherence								
Poor	1.34 (1.16-1.54)	0.0001	1.30 (1.12-1.50)	0.0004	1.48 (1.30-1.68)	0.0000	1.37 (1.20-1.56)	0.0000
Excellent	1.00		1.00		1.00		1.00	

Discontinuation of NNRTI treatment (all cause)

For these analyses only patients who had at least one follow-up visit were included (n=3801). Four hundred and seventy-three 473 (12.4%) patients discontinued either EFV or NVP for all-cause while under care with 219 discontinuations on EFV (9.8%) and 254 discontinuations on NVP (16.1%). Figure 6.3 shows the Kaplan-Meier estimation of the probability of all-cause discontinuation of NNRTI. At 24 months after starting therapy, 13.9% (95% CI of 12.0-15.8%) were estimated to have discontinued nevirapine compared with 8.7% (95% CI of 7.4 - 10.0%) for efavirenz. Further on at 48 months, the corresponding values were 19.8% (95% CI of 17.4 -22.2%) vs 15.0 % (95% CI of 12.9 -17.1%) and at 72 months 24.3% (95% CI of 21.2 – 27.4%) discontinued nevirapine compared with 18.5% (95% CI of 15.7 – 21.3%).

On univariate analysis, the hazard ratio of all-cause discontinuation of nevirapine compared with efavirenz was 1.53 (95%CI of 1.29 – 1.87), $p<0.0001$. Other factors significantly associated with all-cause discontinuation of NNRTI were female gender HR (95% CI) of 1.48 (1.18-1.76), $p=0.0002$, age <40 years with HR (95% CI) of 1.42 (1.19-1.71), $p=0.0002$ and baseline CD4 below 200 with HR (95% CI) of 1.47 (1.25-1.83). On multivariate analysis, female gender was associated with an adjusted HR (95% CI) of 1.29 (1.03-1.63), $p=0.03$, baseline CD4 count <200 cells was associated with an adjusted HR of 1.49 (1.24-1.79), $p<0.0001$ and age < 40 years was associated with HR of 1.25 (95% CI of 1.03-1.51), $p=0.03$. The adjusted HR (95%CI) for all-cause discontinuation of NVP compared with EFV was 1.36 (95% CI of 1.11 – 1.65), $p=0.003$.

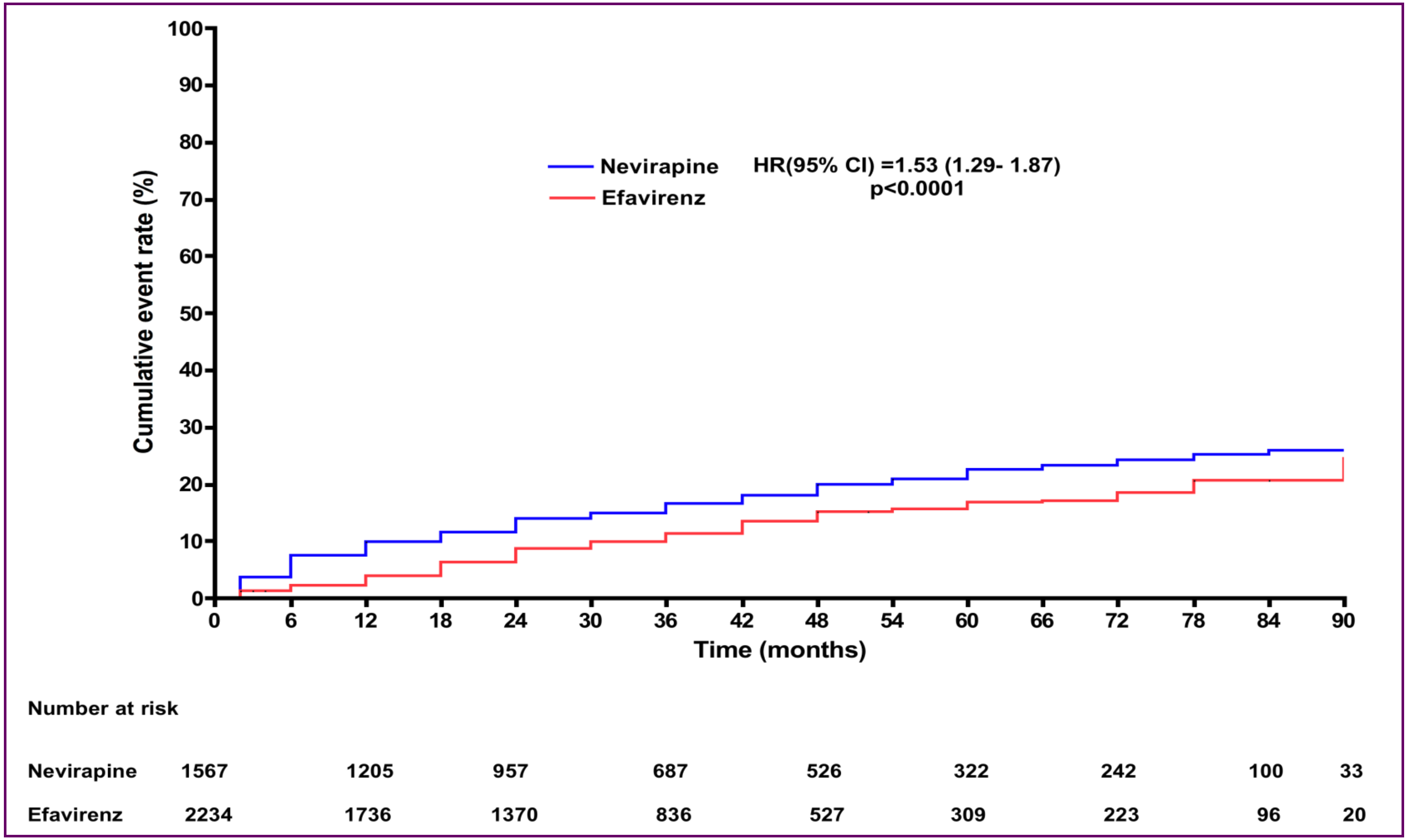


Figure 6.3. Kaplan-Meier analysis of risk of discontinuation of NNRTI for any reason.

*Discontinuation of NNRTI due to clinically or immunologically determined treatment failure***:** Out of the 3,801 patients starting an NNRTI containing ART with at least one follow-up visit, there were 154 (4.1%) discontinuations as result of treatment failure (defined clinically or immunologically) requiring a switch to second line medications. 83 (3.7%, n=2234) patients on efavirenz-containing ART compared with 71 (4.5%, n=1567) patients on nevirapine-containing ART discontinued therapy due to treatment failure. The median time to discontinuation of NVP-containing ART due to treatment failure of 30 months (range 6-78 months) was significantly longer compared with 24 months (range 6-78 months) of patients on EFV-containing ART, p <0.0001 (Wilcoxon signed rank test). The unadjusted hazard ratio of discontinuing a NVP-containing ART compared to an EFV-containing ART regimen as a result of therapy failure was 1.03 (95% CI of 0.75 to 1.42), p=0.84 (shown in Figure 6.4). After adjusting for gender, CD4 count, WHO clinical stage and NRTI backbone, the risk of treatment failure indicated discontinuation of nevirapine compared with efavirenz was not significantly different, HR of 1.21 (95% CI of 0.85 to 1.73), p=0.28.

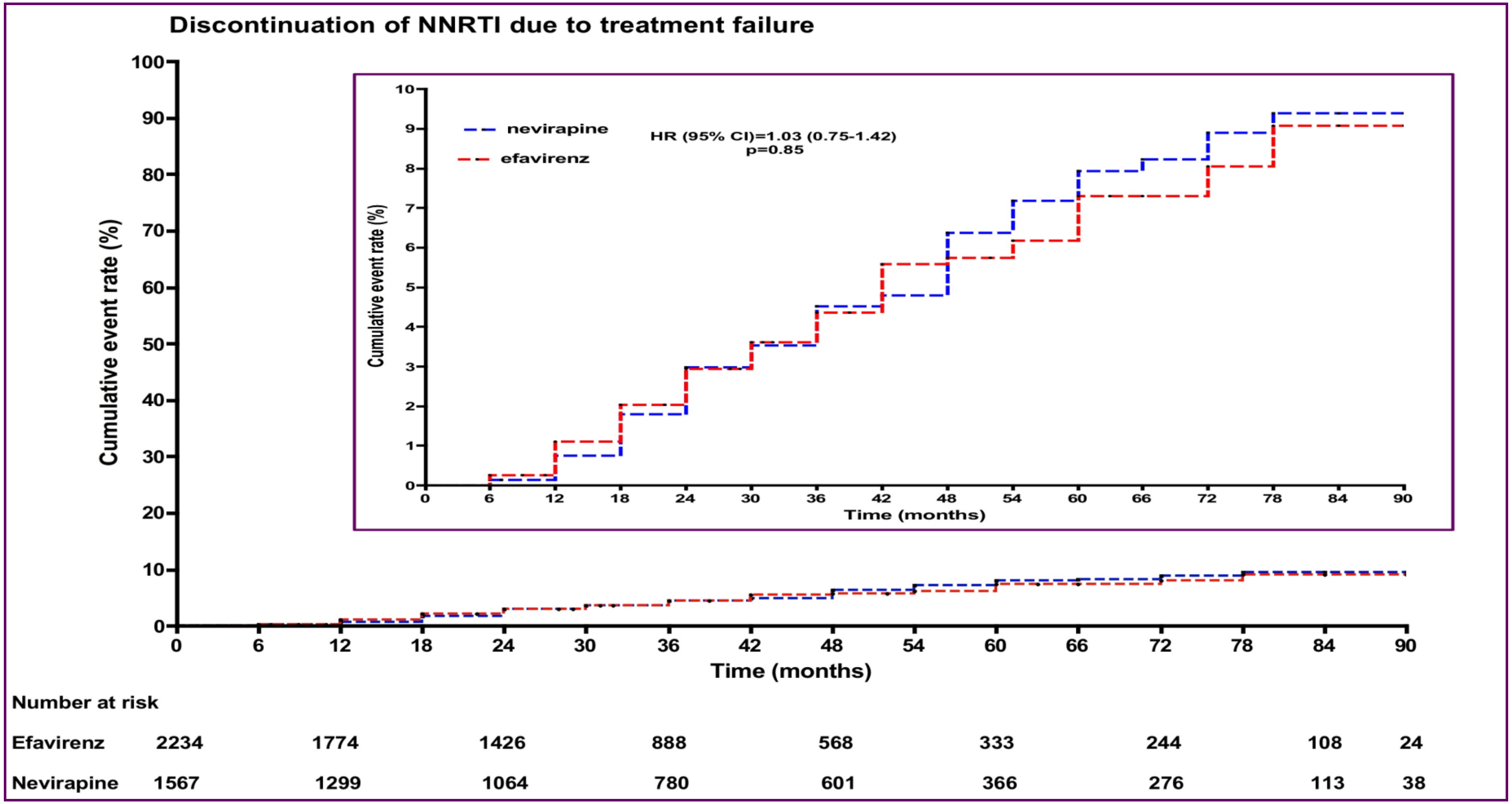


Figure 6.4. Kaplan-Meier risk of discontinuation of NNRTI due to therapy failure.

Discontinuation of NNRTI due to toxicity: There were 61 (3.9%, n=1567) discontinuations of nevirapine compared with 37 (1.7%, n=2234) discontinuations of efavirenz due to drug related toxicity. The median time to discontinuation of nevirapine was 2 months (range, 2 to 56 months) compared with efavirenz of 12 months (range, 2 to 48 months), p=0.0028 (Mann-Whitney's U-test). The unadjusted hazard ratio of discontinuation of nevirapine compared to efavirenz as a result of toxicity was 2.27 (95%CI, 1.55 to 3.46), p<0.0001. In a multivariate Cox proportional model, the risk of discontinuation of nevirapine compared with efavirenz on account of toxicity was 1.89 (1.22-2.92), p=0.004 after adjusting for gender, CD4 count, WHO clinical stage and NRTI backbone. Also, after adjusted analysis females did not have a significantly higher risk of discontinuing NNRTI on account of toxicity compared to males in this model.

Discontinuation of NNRTI due to other reasons: 120 patients discontinued nevirapine and 99 patients discontinued efavirenz due to reasons other therapy failure or drug-related toxicity. The other reasons were discontinuations of nevirapine included, (a) substitution for efavirenz to prevent drug interaction with rifampicin in patients on concurrent anti-tuberculous therapy (n=35), (b) shortage of nevirapine supply (n=6) and (c) no recorded reasons (n=79). Similarly, the other reasons for discontinuations of efavirenz were, (a) substitution for a protease inhibitor due to the fact that patients had HIV-1/2 dual infection (n=2), (b) females got pregnant while taking efavirenz (n=19), (c) females in their reproductive age who wished to change efavirenz because they were preparing for conception (n=3) and (d) no recorded reason (n=75). Compared with efavirenz-based cART, patients initiating nevirapine-based cART had an unadjusted HR of 1.56 (95% CI of 1.22 to 2.05, p=0.0005) of discontinuation for other reasons but an adjusted HR of 1.22 (0.92-1.61), p=0.14. The factors which remained significant upon adjusted analysis were female gender and age<40 years.

Subset analysis of primary outcome measure to examine for backbone effect:

Efavirenz vs nevirapine on a backbone of stavudine plus lamivudine: On adjusted analysis with the Cox proportional hazards regression model, the hazard ratios of failure on nevirapine compared with efavirenz were 1.03 (0.87-1.22) and 0.95 (0.84-1.08) using either a missing= censored or missing = failure approach respectively and similarly, the adjusted odds ratios were 1.09 (0.89-1.33) and 0.91 (0.76-1.11) respectively using the multiple logistic regression. Thus on a backbone of D4T plus 3TC, there were no significant differences in the risk for failure on either NVP or EFV.

Efavirenz vs nevirapine on a backbone of zidovudine plus Lamivudine: On adjusted analysis with the Cox proportional hazards regression model, the hazard ratios of failure on nevirapine compared with efavirenz were 1.20 (1.00-1.43), p=0.05 and 1.02 (0.88-1.19), p=0.76 using either missing= censored or missing = failure approach respectively and similarly, the adjusted odds ratios were 1.31 (1.06-1.62), p=0.01 and 1.05 (0.85-1.28), p=0.67 respectively using the multiple logistic regression suggesting a difference in favour of EFV over NVP on a backbone of AZT plus 3TC .

Secondary end-point analyses:

CD4 T-cell count changes over time

The median (IQR) CD4 counts of patients initiating EFV-based ART of 127 (45 – 212 cells/mm^3, n=2354) was significantly lower than those initiating NVP-based ART of 141 (56 – 221 cells/mm^3, n=1615), p=0.003. Amongst both patient groups similar trends in CD4 increases were also observed as shown in Figure 6.5. Briefly, the changes in the median CD4 counts in the EFV-based ART group compared to the NVP-based group were 279 (171 – 421, n=1090) vs 284 (177 – 419, n=975), p=0.90 at 24 months, 370 (245 – 520, n=469) vs 359 (232 – 543, n=583), p=0.81 at 48 months, 390 (245 – 556, n=277) vs 377 (236 – 558, n=377), p=0.64 at 60 months, 436 (307 – 633, n=211) vs 418 (241 – 581, n=289), p=0.13 at 72 months and 513 (367 – 674, n=26) vs 546 (378 – 749, n=43), p= 0.75 at 90.

In a Generalised linear mixed effects model, the changes in CD4 counts of patients on d4T-containing cART were generally higher than those on AZT-containing cART with slight differences in slopes according the NNRTI-base as shown in Figure 6.6. The slope of CD4 change on d4T compared with AZT was 0.08 cells/mm^3/month (standard error of 0.003), p<0.0001 whilst that for EFV compared with NVP increased at a rate of 0.003 cells/mm^3/month (standard error of 0.004), p<0.0001. Other factors associated with CD4 changes on cART were gender, age and WHO stage with greater increases in females compared to males, decreasing age and earlier WHO clinical stages.

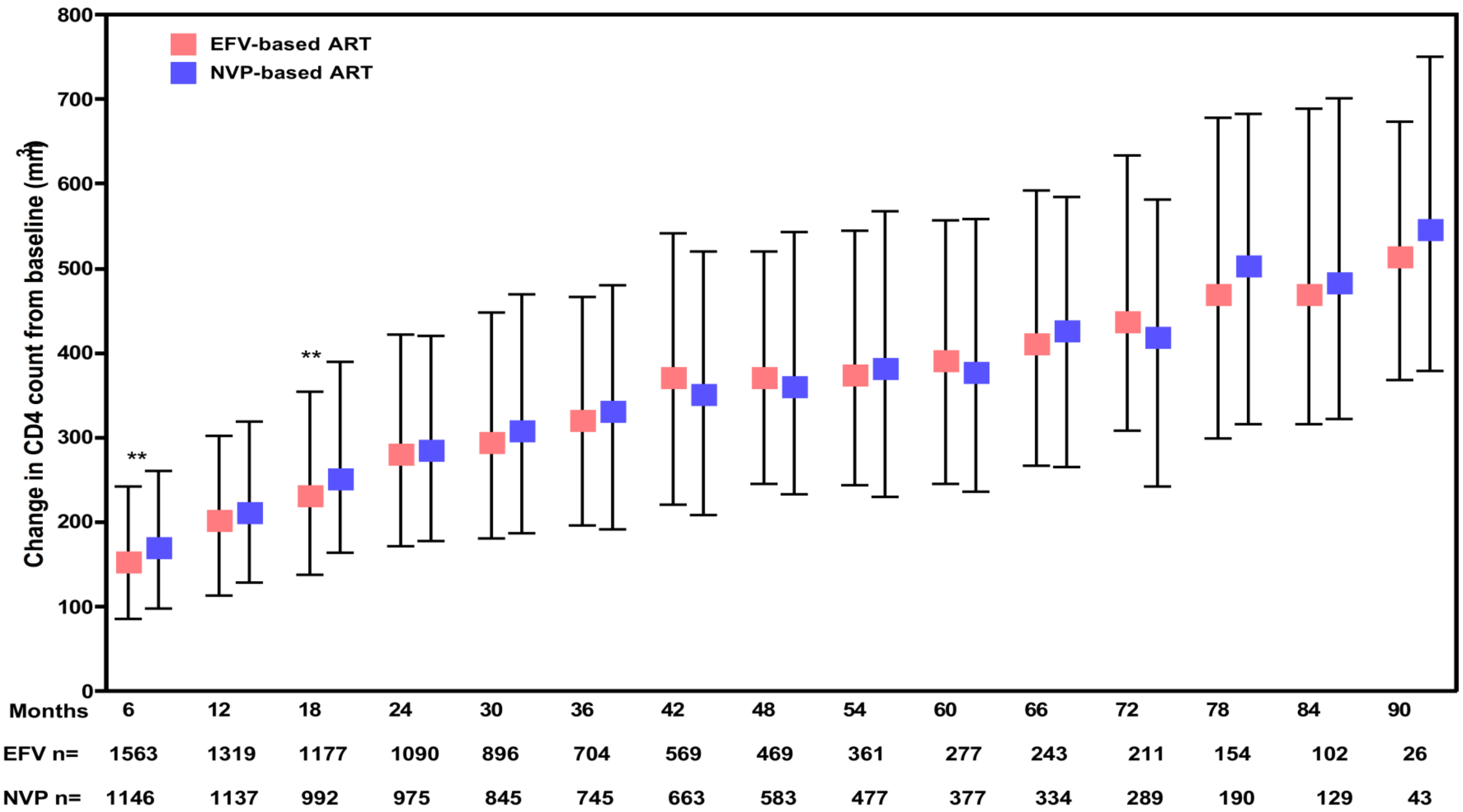


Figure 6.5. Comparison of median (IQR) of change in CD4 from baseline in patients initiating either EFV-based vs NVP-based cART. Double asterixes are for statistically significant difference of <0.01 by Mann-Whitney U-test.

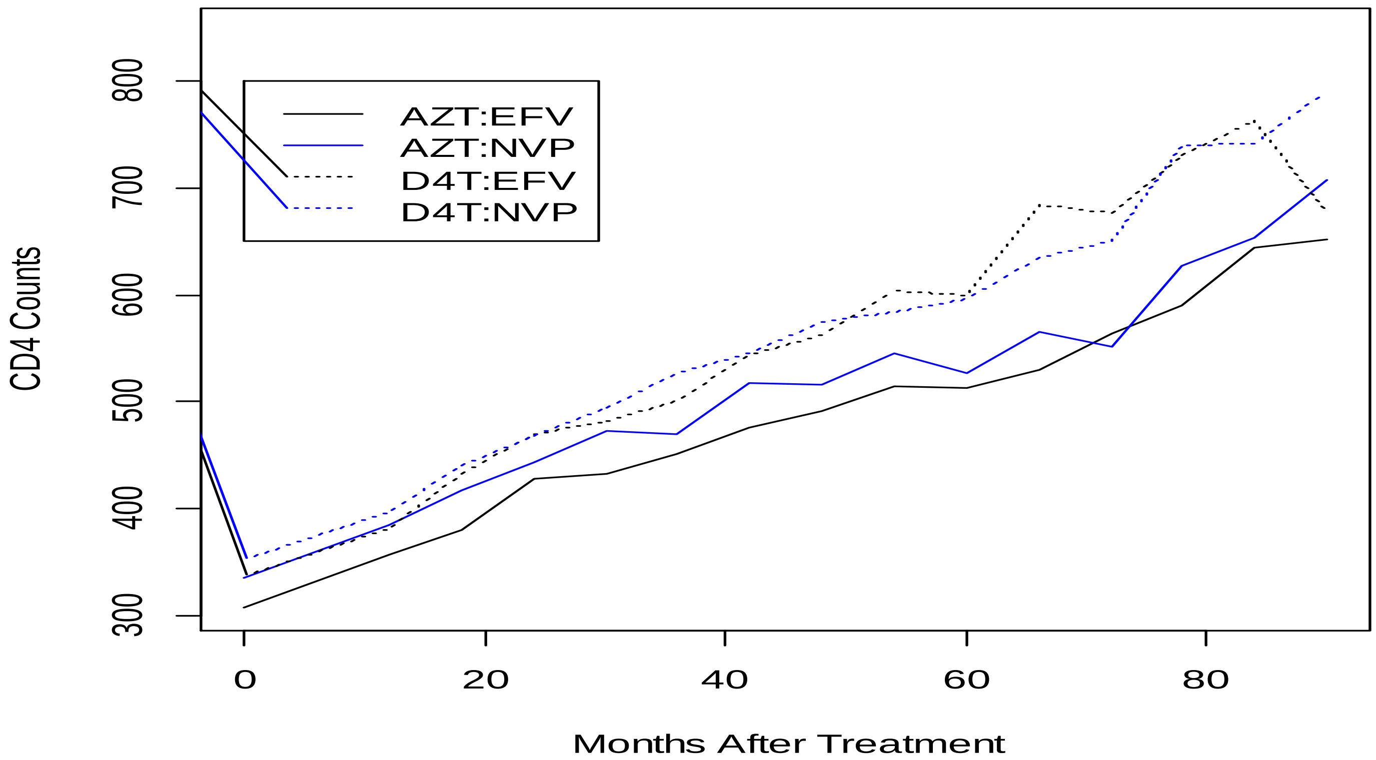


Figure 6.6. Generalised linear mixed effects model of changes in CD4 counts with time on cART. AZT:EFV= zidovudine + lamivudine + efavirenz; AZT:NVP= zidovudine + lamivudine + nevirapine; D4T : EFV = stavudine + lamivudine + efavirenz; D4T:NVP= stavudine + lamivudine + nevirapine.

Body mass index changes over time

The median (IQR) BMI of patients starting EFV-based of 19.6 (17.4 – 22.3 kg/m^2, n=2307) was significantly lower than 19.9 (17.6 – 23.0kg/m^2, n=1595) of those starting an NVP-based ART, p=0.002. As shown in Figure 6.7, a pair-wise comparison of median BMIs on treatment showed that patients on NVP were more likely to have a higher BMI than those on EFV. In a linear mixed effects model, changes in BMI were comparably higher amongst patients on NVP than those on EFV with a backbone of AZT plus 3TC (Figure 6.8A). However on a backbone of D4T plus 3TC, non-significant differences in BMI changes over time were identified for patients on either NVP or EFV (Figure 6.8B). Also BMI changed by 0.57kg/m^2/month (standard error, [SEM] of 0.16) faster amongst females compared with males, p=0.0004 and compared with patients with WHO stage 4 at baseline, the slope of BMI changes amongst patients with WHO clinical stages 3, 2 and 1 were -0.59 (SEM of 0.18, p=0.001), -0.79 (SEM of 0.26, p=0.002) and -0.92 (SEM of 0.31, p=0.003).

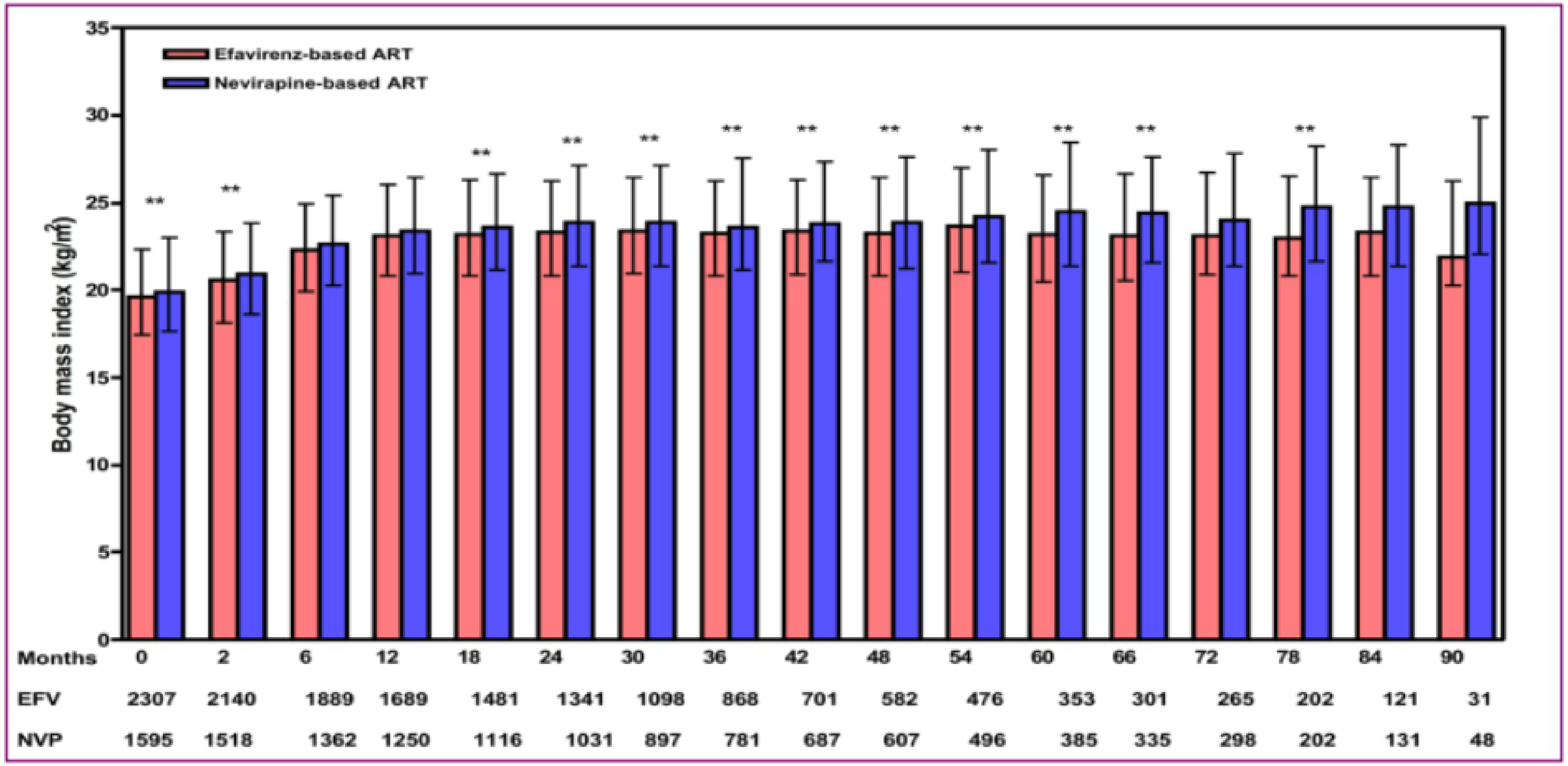


Figure 6.7. Changes in BMI on either efavirenz or nevirapine-based cART. Each rectangle and bar represents median and Interquartile range.

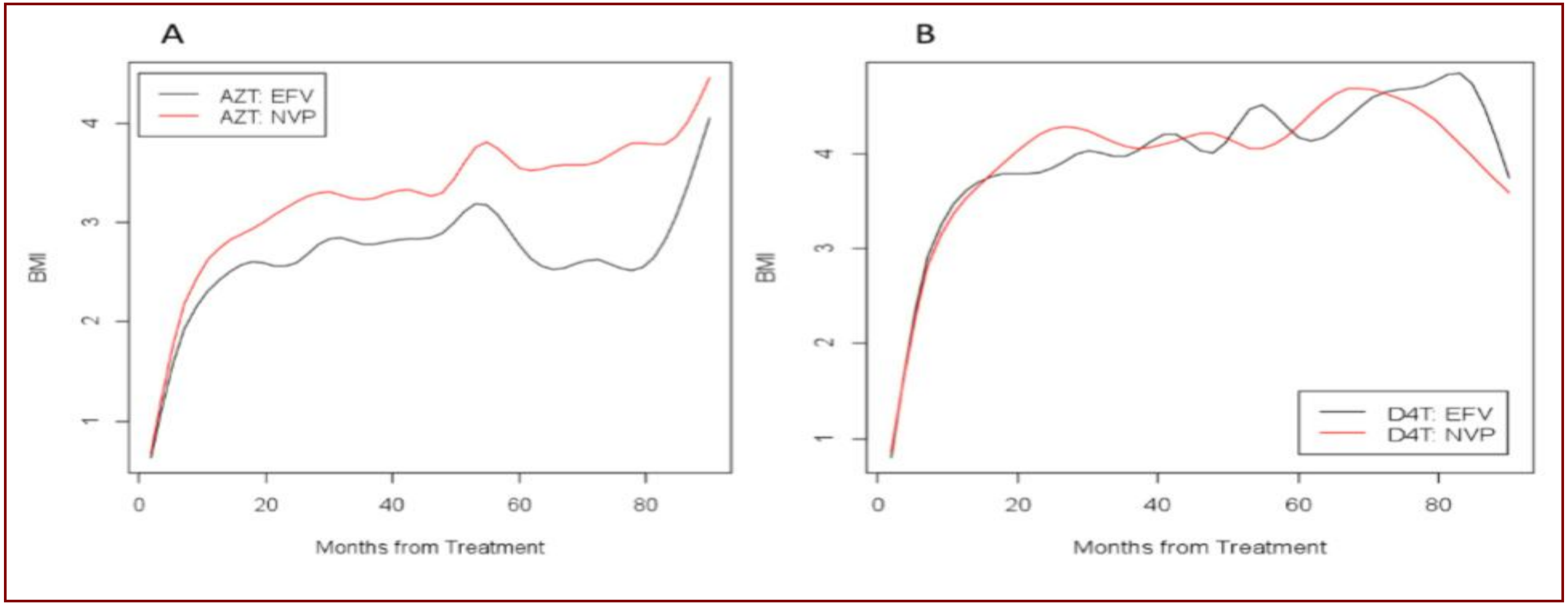


Figure 6.8. A linear mixed effects model of changes in BMI with time on cART. (A) compares EFV vs NVP on an AZT + 3TC backbone and (B) compares EFV vs NVP on a D4T + 3TC backbone.

6.4 Discussion

For millions of patients with HIV-1 infection in Sub-Saharan Africa antiretroviral therapy is initiated with a combination of a non-nucleoside reverse transcriptase inhibitor-base of either efavirenz or nevirapine with a backbone of two nucleoside reverse transcriptase inhibitors commonly zidovudine plus lamivudine or stavudine plus lamivudine within national ART treatment programmes. The choice of the NNRTI is driven by clinical indications, physician and or patient preferences, availability of medications and ability to monitor for the development of adverse events. This study shows that under these settings, the long-term clinical and immunologic treatment outcomes are comparable whether efavirenz or nevirapine is chosen to initiate therapy. The primary composite outcome measure comprising of deaths, clinical progression and discontinuation of NNRTI for all-causes showed that compared with efavirenz, the adjusted hazards and odds ratios of therapeutic failure for nevirapine were 1.20 (0.97 to 1.49), p=0.10 and 1.09 (0.79 to 1.49), p=0.61 respectively. There were no discernible differences in the risks for death and disease progression between both group treatment groups on adjusted analyses. There was however a divergence in the risk for all-cause discontinuation of NNRTI use with a 36% (95% CI of 11% to 65%), p=0.003 higher risk of discontinuation of nevirapine compared with efavirenz in adjusted analysis. The dominant reason for this observed difference in NNRTI discontinuation was due to the 89% (95% CI of 22% to 192%) higher risk of nevirapine causing treatment limiting toxicity compared with efavirenz, p=0.004.

As discussed in Chapter 5 of this dissertation, compared to efavirenz, patients on nevirapine were more likely to discontinue therapy on account of treatment-limiting skin rash or severe to life threatening hepatotoxicity while efavirenz was more likely to be discontinued on account of severe central nervous system adverse events. In various studies, the frequencies of severe grade 3 or 4 rash have varied widely with 3.4% among patients on nevirapine twice daily dose in the 2NN study[4], 7% in the ATLANTIC study[517] and 4% in the INCAS study[518]. Again, there were more treatment discontinuations for liver-associated laboratory abnormalities among patients on nevirapine than efavirenz, although the frequencies of grade 3 and 4 elevations in liver transaminases were comparable for both efavirenz and nevirapine (Chapter 5). These results show that overall, efavirenz was better tolerated by patients than nevirapine due to toxicity in this cohort with two fatal cases of nevirapine-associated Stevens Johnsons syndrome from nevirapine.

The most significant period for adverse treatment outcomes are within the first six months after initiation of treatment. During this period nearly 50% of all deaths, loss to follow ups, treatment discontinuations due to toxicities and disease progression were observed. The risk of disease progression was comparable among patients on either nevirapine or efavirenz. The major determinants of further AIDS defining events on treatment were the well established baseline risk factors such as starting with a low CD4 count, low body mass index and having advanced disease at baseline while the most significant on-treatment factor identified in association with clinical progression was poor adherence. The commonest amongst the AIDS defining events that occurred was tuberculosis, for which reason treatment of nevirapine had to be substituted for efavirenz to allow for treatment of tuberculosis with anti-tuberculous medications due to the complex interactions that exists between rifampicin and nevirapine[515,516]. This interaction is such that co-administration of rifampicin with nevirapine leads to sub-therapeutic exposure of nevirapine with attendant risk of loss of virologic suppression.

Of note a number of patients who developed disease progression were lost to follow-up after these events were diagnosed and treatments for these opportunistic infections were initiated. Loss-to-follow up is a common outcome or confounder in most HIV antiretroviral therapy cohorts in SSA where cART is initiated when patients have advanced HIV disease. The risk factors identified for loss-to-follow up in this cohort has been discussed in Chapter 4. Loss-to-follow up was not included as an outcome measure in the primary analysis because although patients who withdrew from the programme may inevitably experience a deterioration of their clinical status without cART, such an adverse outcome measure could not be directly attributed to a failure of therapy per se. However, it is conceivable that drug toxicities may engender loss-to-follow up which could be a medication-associated reason for withdrawal from the treatment programme. Conversely, most patients lost to follow up were found to have died in a survey of 210 patients out of the 934 patients who were lost to follow-up in the present cohort (Chapter 3) which suggests the possibility that withdrawal from care may be due to either unreported deaths from disease progression, fatal toxicities or migration to other treatment centres. Either way, the inclusion of loss-to-follow up as an outcome measure in sensitivity analyses where missing was treated as a failure yielded similar outcomes in both the Cox and logistic regression models with the adjusted risk of treatment failure of 0.97 (0.82 to 1.15) and 0.98 (0.77-1.24), p= 0.73 and 0.83 respectively comparing efavirenz with nevirapine.

Deaths were mainly recorded during the very proximal periods of follow up and were determined predominantly by the severity of baseline immunosuppression and by advanced clinical disease. Interestingly, the state of immunosuppression measured by CD4 T-cells counts and clinical stage of disease were independent and non-interactive factors in predicting each of the components of the composite measures of treatment failure (data not shown). When the cause of death was known, it was more frequently due to tuberculosis. Indeed, the relatively disproportionate numbers of patients who started efavirenz compared to nevirapine in this cohort was because of concurrent anti-tuberculous medications which these patients were taking when cART had to be started. This is probably one of the reasons for the significantly lower CD4 counts among patients who initiated efavirenz compared with nevirapine, median of 127 vs 140 cells/mm^3 respectively, p=0.004. However during follow up, CD4 responses were robust among survivors in both treatment groups over time (Figures 6.5 and 6.6) and were accompanied by positive changes in body mass index (Figure 6.7). In the generalised linear mixed model, the slope of CD4 changes over 90 months follow up were comparable overall, although there was a statistically significant higher rate of increase among patients on efavirenz compared with nevirapine at a rate of 0.003 cells/mm^3/month. In addition patients on a backbone of D4T plus 3TC increased CD4 counts at a faster rate compared with those on AZT plus 3TC, indicating that the backbone selected for initiating cART had an impact on CD4 recovery. It is to be recognised though that this difference was statistically significant by virtue of the large sample size and may be minimal clinical relevance. However, it is noteworthy that in a subset analysis where the two NNRTIs were compared on either an AZT plus 3TC or D4T plus 3TC backbone, patients on an AZT+3TC backbone with efavirenz had a 20% lower adjusted hazard and 31% lower adjusted odds of composite treatment failure (missing= censored analysis) compared with those on nevirapine, p=0.05 and 0.01 respectively but no such differences were observed on a D4T+3TC backbone. Thus there appears to be subtle differences in risk of treatment failure of NNRTI dependent on the NNRTI backbone as has been previously shown by Annan et al[22].

To a large extent switching to second line therapy due to treatment failure was determined using immunological criteria. The risk of immunologically determined treatment failure warranting therapy change to second line were comparable in both groups with an adjusted hazard ratio of 1.03 (95% CI of 0.75 to 1.42), p=0.84 among nevirapine (71 patients) compared with efavirenz (83 patients). Because immunological failure was determined after at least one year of cART, there is a potential risk for development of virological falure with emergence of resistance strains and studies are proposed to answer these questions in the future.

There is some difficulty in comparing this data with those of other cohort studies because whereas the effectiveness of the efavirenz- or nevirapine-based cART has been compared using predominantly clinical and immunological outcomes over the long-term under routine care in a programme setting, the previously reported cohorts have focused on short-term virologic and immunologic outcomes with somewhat limited clinical outcome measures due to the relatively short durations of follow-up. For instance, in a cohort of 888 patients, Matthews and colleagues found a hazard ratio for therapy failure at 24 weeks of 2.03 (1.26 to 3.18) for patients starting nevirapine-based cART compared with those starting an efavirenz-based cART[18]. Again, in a study by Keiser and co-workers[19], involving 1,078 patients, efavirenz (n=555) was found to be superior to nevirapine (n=523) with regard to time to virologic failure and the proportion of patients with HIV-1 RNA below the detection limit of <400 copies/mL over 192 weeks of observation. Similar results were observed in the Italian cohort of naïve HIV patients[519] (n=694) and also in the South African cohort[8] (n=2,817) with adjusted hazards ratios of virologic failure of 2.08 (1.37-3.15) and 1.52 (1.24 to 1.86) respectively. Annan and colleagues[5] found among a British cohort of 994 anti-retroviral naïve patients that the two NNRTIs were comparable in their 6-month time to virological success: efavirenz 71%, nevirapine 72%, p=0.77. In a more recent study, involving 14,857 from North American and European cohorts in the HIV-CAUSAL collaboration[20], efavirenz was shown to be associated with lower mortality, lower incidence of AIDS-defining illnesses, a larger increase in CD4 count at 12 months and a smaller risk of virologic failure at 12 months compared with nevirapine[20]. Virologic end-points admittedly are probably the most sensitive surrogate markers of treatment efficacy in HIV treatment due to its ability to predict long-term clinical and immunological events which this study captures due to its considerable length of follow up and the large number of patients compared with previous cohorts.

The choice of treatment efficacy end-points has varied among different observational and experimental studies. In the 2NN study, therapy failure was defined as a composite of deaths, withdrawals, treatment discontinuations and virologic failure. In that large randomised controlled trial, the treatment efficacy end-points were chosen to compare efavirenz with nevirapine with the overall goal that for first line cART to be effective and durable, they must have minimal toxicity to have the greatest chance of achieving viral suppression. Under these experimental conditions, nevirapine administered twice daily was found to be non-inferior to efavirenz, the difference between nevirapine and efavirenz was 5.9% (95% CI of -0.9 to 12.8%). The end-points for this study were chosen to approximate those of the 2NN study[4] but of course without viral load outcomes and randomisation. On its own merit, although this study lacks the elegance of virologic end-points in the assessment of therapeutic efficacy, the outcome measures chosen assess the effectiveness of these NNRTI by comparing tolerability, clinical and immunological outcomes. Again, very limited exclusions were imposed in selecting patients for this analysis in order to compare the treatment effect of these 2 medications as they are used in under field settings. Further the composite outcome measures were analysed using both Cox proportional hazards and multiple logistic regressions with sensitivity analyses to minimize confounding but in all these analyses, the two medications were comparable in the composite outcomes. Finally, with the exception of treatment discontinuations due to toxicity, nevirapine was comparable to efavirenz in the risks for death, disease progression and proportion with loss to follow-up.

There are a number of limitations to this study worth noting. The analysis was based on data from a retrospective observational cohort study and is subject to all the biases that are inherent with cohort studies. Although many biases can be accounted for in adjusted analysis, there may still be unmeasured confounders that could not be accounted for. This is because unlike randomised controlled trials, cohort studies are prone to biases such as confounding by indication or some other factors which may be difficult to exclude. For instance, a higher proportion of patients prescribed nevirapine-based cART were females (84.7%) compared with males (15.3%) in order to avoid the potentially teratogenic effects of efavirenz in a predominantly young female population in their reproductive age, which introduced some selection bias. However two robust methodologies namely Cox proportional hazards regression and multiple logistic regression were used to examine the primary outcome measure with sensitivity analyses performed with the aim of minimizing confounding. The composite end-point had as a component treatment discontinuations due to other reasons such as non-availability of medications which led to permanent substitutions even though the limited medications became available later on. Also, as it is evident from Chapter 5, although grade 3 and 4 hepatic enzyme elevations were of comparable frequencies in both efavirenz and nevirapine users, physicians were more likely to change nevirapine for this event. Thus the chosen composite outcome measure had a component which could be influenced by patient management by clinicians. Again, some treatment discontinuations were not explained because reasons for discontinuations were not always recorded in patient's folders which could potentially introduce information bias. Data on teratogenicity among females who became pregnant on efavirenz were not available in the patients' records. The findings from this study are based on observations from a single treatment site in West Africa where although uniformity in level of care is expected, there could be differences in treatment practises emerging because clinicians providing care for HIV patients had different levels of expertise ranging from physician specialists, medical officers, nurses and allied health workers in a busy out-patient clinic which could influence the quality clinical decision making. This notwithstanding, consultations among health workers are routinely held during clinics to assure standard practises. Thus said, it should be noted that the main aim of this study was to compare efavirenz with nevirapine under operational conditions in programmatic settings in Sub Saharan Africa where resources are indeed limited with heavy clinics. Clearly, the decision to select one NNRTI over the other or change therapy due to adverse effects in a cohort study such as this present one is subject to a selection bias driven by the physicians' preconceived ideas about efficacy and safety of therapy regimen.

In conclusion, under programme settings as it pertains in most resource constrained settings, there were no significant differences in the long-term clinical and immunological outcomes among patients initiating either efavirenz- or nevirapine-based cART in this large Ghanaian cohort of naïve HIV-patients. Nevirapine however was more likely to be discontinued for adverse toxicity compared with efavirenz. Certainly, this cohort has been set up to study these 2 NNRTIs over the long-term with 10-year outcome analyses planned in the next two years (2014). The hope is that viral load monitoring would have become part of the routine care, so that they could be included in these future studies.

CHAPTER SEVEN

A prospective study of tolerability and pharmacokinetic interactions between efavirenz and artemisinin-based antimalarial therapy in Ghanaian HIV patients

Introduction

Human Immunodeficiency Virus (HIV) and *Plasmodium falciparum* malaria have a large geographical overlap in sub-Saharan Africa, causing significant morbidity and mortality and various new therapeutic challenges. Whilst current World Health Organisation (WHO) recommendations on the management of malaria do not differ for HIV patients, few studies have assessed the interactions between antiretrovirals (ARVs) and antimalarials. Indeed a 2004 WHO report identified that urgent research is required into the pharmacodynamic and pharmacokinetic interactions between antimalarials and ARVs[520].

Artemesinins are plant-derived peroxide antimalarials active against the sexual and asexual forms of *Plasmodium sp* and are generally well tolerated, safe and effective (in study populations)[521]. Despite recent concerns over the development of resistance in western Cambodia, artemesinin-based combination therapy (ACT) remains the policy standard first-line treatment for uncomplicated malaria in Africa[522, 523]. The increasing use of artemesinins and increasing access to ARVs (in areas of high HIV prevalence) raises the likelihood of co-prescriptions, yet minimal data exists for the safety and efficacy of ACT in these populations[524]. Many such pharmacokinetic interaction outcomes have been predicted and described but published data remains scarce [343, 525].

Despite WHO recommendation that artemesinins should be combined with a second antimalarial drug with a longer half-life, many countries use artemesinins alone for a variety of reasons including tolerability, supply of alternative drugs and cost. Artesunate (AS) is a commonly used semi-synthetic water-soluble artemesinin which is safe and effective in severe malaria[526]. It is a prodrug which is rapidly biotransformed by Cytochrome P450 (CYP450) 3A4 subfamily to dihydroartemsinin (DHA) with a half-life of 2-5 minutes after intravenous administration[526]. This highly potent active metabolite DHA is eliminated by glucoronidation with a half-life between 40-60 minutes. Artemisinins have been shown to be inducers of CYP3A4 in primary human hepatocytes and have been observed to have clinically significant effects on both CYP3A4 and CYP2B6[527 - 529], even after a single dose. Common adverse effects include nausea, vomiting and gastrointestinal disturbance, whilst more infrequent side effects such as neutropenia, haemoglobinuria and anaemia have been previously reported[530]. Neurotoxicity has been proven both *in vitro* and in experimental animal studies, in which there was selective damage to brain stem nuclei[531, 532]. The significance of this neurotoxicity in humans is unclear however isolated case reports and one case series have attributed hearing loss[533], cerebellar dysfunction[534] and tremor[535] to artemesinins but this has not been validated in further neurophysiological studies or neuropathological studies[536, 537]. One of the few studies published on artemisinin drug interactions with antiretrovirals looking at artesunate PK (in combination with amodiaquine) in patients taking efavirenz was terminated early due to hepatotoxicity in several patients [345, 538, 539].

The NNRTI efavirenz, widely employed in WHO first line regimens, is primarily metabolized by CYP2B6 and is known to induce CYP3A4 [276, 343]. These common metabolic pathways mean a significant interaction is likely which may compromise both the safety and efficacy of either the antiretroviral or the antimalarial. Under randomized trial conditions efavirenz has been found to be safe and well tolerated. However it is not certain whether a pharmacokinetic interaction between efavirenz and co-administered artesunate could potentiate side effects of either medication and whether this will influence their efficacy. In Ghana artesunate is sometimes used as a single antimalarial agent providing an opportunity to study this drug alone when co-prescribed with efavirenz. The aims of this study were to determine the effect of steady state therapy with efavirenz on artesunate/DHA pharmacokinetics, to investigate the effect of artesunate on steady state concentrations of efavirenz and to evaluate the safety and tolerability of artesunate co-administered with efavirenz by assessing symptoms or laboratory features of toxicity of artesunate/DHA and efavirenz and to evaluate other surrogate markers for reduced drug levels of antimalarial such as failure of malarial parasite clearance - early treatment failure - in those with positive blood films.

Methods

Please refer to chapter 2 section 2.7

Results

Baseline demographic, clinical and laboratory features of study participants at recruitment.

Twenty-five (25) HIV-infected patients and 21 patients whose HIV sero-status (referred to as controls from hence) was unknown were recruited into the study. Three HIV-infected patients were excluded because they did not complete the study by missing day 5 visits. The HIV-infected patients were significantly older than control patients, median age of 49 years (range 27-67) compared with 25 years (range 17-45), p<0.05. Seventeen (17) HIV-infected patients were females while 12 of the controls were females with no significant differences in gender distribution. Among the HIV-infected patients, all were on a nucleoside reverse transcriptase inhibitor backbone of zidovudine plus lamivudine except two patients who were on stavudine plus lamivudine. The median duration on cART for the HIV-infected patients was 26.5 months (range of 1.25 to 50.75 months) with a median CD4 count at the time of recruitment into study of 407 (range of 38 to 1035 cells/mm^3).

Table 7.1 shows the frequencies of clinical symptoms of both HIV-infected and controls at recruitment for suspected malaria with headache, fever and sleeping difficulties often the commonest complaints. The HIV-infected group presented after having experienced symptoms for a median of 7 days (range 1 to 14 days) compared with 3 days (range of 1 to 7 days) for the control group, p=0.01. A significant proportion of HIV-infected patients on efavirenz-based cART reported having nightmares compared with control group while significantly more among the control group admitted to having nausea and vomiting compared to the HIV-infected group. Clinical examinations were not remarkable for both groups of patients except for 6 patients who had pyrexia with temperature above 37.8^{0}C, 5 in the control group and 1 HIV-infected group. There were several dissimilarities in the haematology and serum biochemistry results between the two groups as shown in Table 7.2. Briefly, HIV-infected patients had significantly lower haemoglobin concentrations with higher mean corpuscular red cell volume and haemoglobin and consequently wider red cell distribution width compared with control patients. HIV-infected patients had significantly lower total white cell and neutrophil counts as well as lower serum creatinine, albumin and total bilirubin concentrations than controls. Only 4 controls and 1 HIV-infected patient had malaria parasites on slide examination with a geometric mean level of parasitemia of 3,312/μl (95% CI of 314.6 to 34,866/μl; range of 255 to 39,300).

Table 7.1. Frequency of symptoms of malaria and potential toxicity of efavirenz or artesunate among HIV-infected and control patients.

Symptoms	Number of patients reporting symptoms				Fisher's exact test			
	HIV+ (n=22)	HIV+	HIV- (n=21)	HIV-	p-value[1]	p-value[2]	p-value[3]	p-value[4]
	Day 1	Day 5	Day 1	Day 5				
Chest pain	1	1	2	0	ns	ns	ns	ns
Palpitations	6	4	4	0	ns	ns	ns	ns
Dizziness	7	2	6	0	ns	ns	0.02	ns
Blackouts	0	0	0	0	ns	ns	ns	ns
Shortness of breath	1	4	2	0	ns	ns	ns	ns
Fever	20	0	16	0	<0.0001	ns	<0.0001	ns
Headache	21	2	17	3	<0.0001	ns	<0.0001	ns
Weakness of arms/legs	2	1	5	0	ns	ns	0.05	ns
Difficulty walking	1	0	0	0	ns	ns	ns	ns
Difficulty sleeping	12	6	7	0	ns	ns	0.009	0.02
Nightmares	9	3	2	0	ns	0.03	ns	ns
Difficulty concentrating	0	0	0	0	ns	ns	ns	ns
Suicidal thoughts	0	0	0	0	ns	ns	ns	ns
Visual disturbance	2	2	1	2	ns	ns	ns	ns
Speech disturbance	0	0	0	0	ns	ns	ns	ns
Hearing problems	2	1	0	0	ns	ns	ns	ns
Fits/ faints	0	0	0	0	ns	ns	ns	ns
Numbness/pins	9	4	7	1	ns	ns	0.04	ns
Tremor	0	1	0	0	ns	ns	ns	ns
Diarrhoea	0	0	2	0	ns	ns	ns	ns
Nausea and vomiting	0	1	5	1	ns	0.02	ns	ns
Rectal bleeding/ melena	0	0	0	0	ns	ns	ns	ns
Jaundice	0	0	0	0	ns	ns	ns	ns
Pruritus	2	2	2	0	ns	ns	ns	ns
Skin rash	1	1	1	0	ns	ns	ns	ns

[1] compares proportion with specific symptoms among HIV-infected patients before and after course of Artesunate (day 1 vs day 5); [2] compares HIV-infected and non-infected patients before course of Artesunate (day 1); [3] compares HIV-non-infected patients before and after course of Artesunate (day 1 vs day 5); [4] compares HIV-infected and non-infected patients after course of Artesunate (day 5). Parenthesis refers to nervous system symptoms.

Table 7.2. The haematology and serum biochemistry results among HIV-infected and non-infected controls before and after a 5-day course of artesunate 200mg twice daily for clinically suspected malaria.

Variable	**HIV patients median (range)**	**Controls median (range)**	**HIV patients median (range)**	**Controls median (range)**	**p-values[1]**	**p-values[2]**	**p-values[3]**
	Day 1	Day 1	Day 5	**Day 5**			
Haemoglobin concentration (g/dl)	11.1 (4.5 – 13.5)	12.7 (10.8-16.4)	10.5 (4.1 -12.9)	11.7 (9.8 – 15.6)	<0.0001	0.29	0.28
Mean corpuscular volume (fl)	106.5(90.1-120.1)	85.8 (75.7 – 93.9)	107.3(90.9-122.9)	85.6(75.4-94.4)	<0.0001	0.86	0.76
Mean corpuscular haemoglobin (pg)	36.8 (30.0 – 41.4)	27.9 (22.7 - 31.7)	37.1 (29.6-42.0)	27.3 (24.5-31.7)	<0.0001	0.51	0.75
Red cell distribution width (CV%)	14.4 (13.2 – 34.7)	13.7 (12.6 – 21.0)	14.6 (13.6-21.6)	13.8 (12.6-21.7)	0.009	0.74	0.89
White cell count (x 10^9)	4.3 (2.0 – 12.0)	6.1 (3.6 – 9.8)	4.7 (2.2-7.5)	5.7 (3.1-14.3)	0.0004	0.71	0.21
Neutrophil count (x 10^9)	1.6 (0.7 – 9.7)	3.6 (1.4 – 7.9)	1.9 (1.0-4.7)	2.3 (1.0-10.4)	0.0004	0.92	0.05
Platelet count (x10^{12})	208 (56 – 456)	191 (66 – 371)	211 (67-370)	191 (123 – 423)	0.70	0.63	0.94
Serum alanine transaminase (IU/l)	23 (10 – 49)	13 (7 – 67)	21 (9-64)	13.0 (7.0 – 70.5)	0.09	0.56	0.82
Serum aspartate transaminase (IU/l)	39 (18 – 204)	32 (16 – 79)	32 (14 – 135)	25.0(17.0 – 66.5)	0.05	0.05	0.17
Serum total bilirubin (mmol/l)	7 (2 – 31)	15.0 (5.9 – 63.3)	5 (3 – 26)	14.0 (4.6 – 44.9)	0.0004	0.24	0.56
Serum albumin (g/l)	43 (31 – 48)	46.0 (25.5 – 51.7)	42 (29 – 46)	45.5 (25.5-51.7)	0.05	0.21	0.67
Serum creatinine (μmol/l)	64 (31 – 130)	93.5 (56.0 – 132.0)	68.5 (48.0-155.0)	93.5(56.0-132.0)	0.0006	0.16	0.60
Serum urea (mmol/l)	3.8 (2.4 – 6.6)	2.8 (1.1 – 6.4)	4.0 (1.8 – 7.0)	2.8 (1.1 – 6.4)	0.20	0.99	0.72
Serum sodium (mmol/l)	141 (131 – 149)	138 (128 – 150)	139 (132 – 150)	138 (128 -150)	0.08	0.26	1.00
Serum potassium (mmol/l)	5.1 (3.8 – 9.5)	4.3 (3.9 – 5.6)	4.9 (4.2 – 6.2)	4.3 (3.9 – 5.6)	0.32	0.20	0.01

[1] compares medians at day 1 between HIV-infected patients on efavirenz and patients without known HIV infection (controls).

[2] compares medians at days 1 and 5 of HIV-infected patients after 5-day course of artesunate for clinically suspected malaria.

[3] compares medians at days 1 and 5 of patients with suspected malaria without known HIV infection after 5-day Artesunate course.

Clinical and laboratory results upon completion of 5-day course of artesunate for treatment of clinically presumed malaria: Upon completing a 5-day course of artesunate both groups of patients reported resolution of most of the symptoms presented at baseline particularly the proportions with headaches and fevers as shown in Table 8.1. Anti-malarial therapy was well tolerated with no adverse side effects in both groups. However, among the HIV-infected group some exceptions are worth noting. First, whereas all control group patients reported resolution of sleeping difficulty on day 5, among the HIV-infected group 6 out 12 who had sleeping difficulty had it persisting with significant differences noted in the proportion of patients with this symptom between the two groups on day 5. Second, nightmares or abnormal dreams resolved in both control patients at day 5 but persisted in 3 out of 9 HIV-infected patients. Third, pins- and –needles possibly suggestive of peripheral neuropathy resolved in all but one control patient but persisted in 4 out of 9 HIV patients with these symptoms after treatment. Thus although the nervous system symptomatology which were present at baseline completely resolved in most control patients, among the HIV-infected patients some had it persisting with non-significant differences at day 5 compared to day 1. Overall, clinical examinations were normal in both groups, with no remarkable alterations in haematological and biochemical parameters on day 5 as well. Also no malaria parasites were observed after therapy in both groups.

Steady-state concentrations of efavirenz: Before initiating artesunate, the median (range) concentration of efavirenz among HIV-infected patients was 2413 (312.9 – 13,060ng/ml) and did not significantly change within hours 1, 4 and 6 on day 1 and also on hour 6 on day 5 with median concentrations of 2136 (264.3 – 10,615ng/ml), 2357 (249.3 – 12,024ng/ml), 2196 (249.3 – 11,118ng/ml) and 1586 (1,216 – 11,822ng/ml) respectively. One patient had sub-therapeutic concentrations of plasma efavirenz on day 1 although he admitted to being adherent and 5 had supra-therapeutic concentrations and the remainder were within therapeutic range. The kinetics of efavirenz is shown in Figure 8.1. The median percentage change in concentration of efavirenz from baseline at times 1, 4, 6 hours on day 1 and on day 5 were -13.5% (range, -29.3% to 121.4%), -14.8% (range, -53.5 to 120.4%), -21.4% (range, -45.4% to 114.9%) and -13.9 (range, -35.9 to 134.7%) respectively.

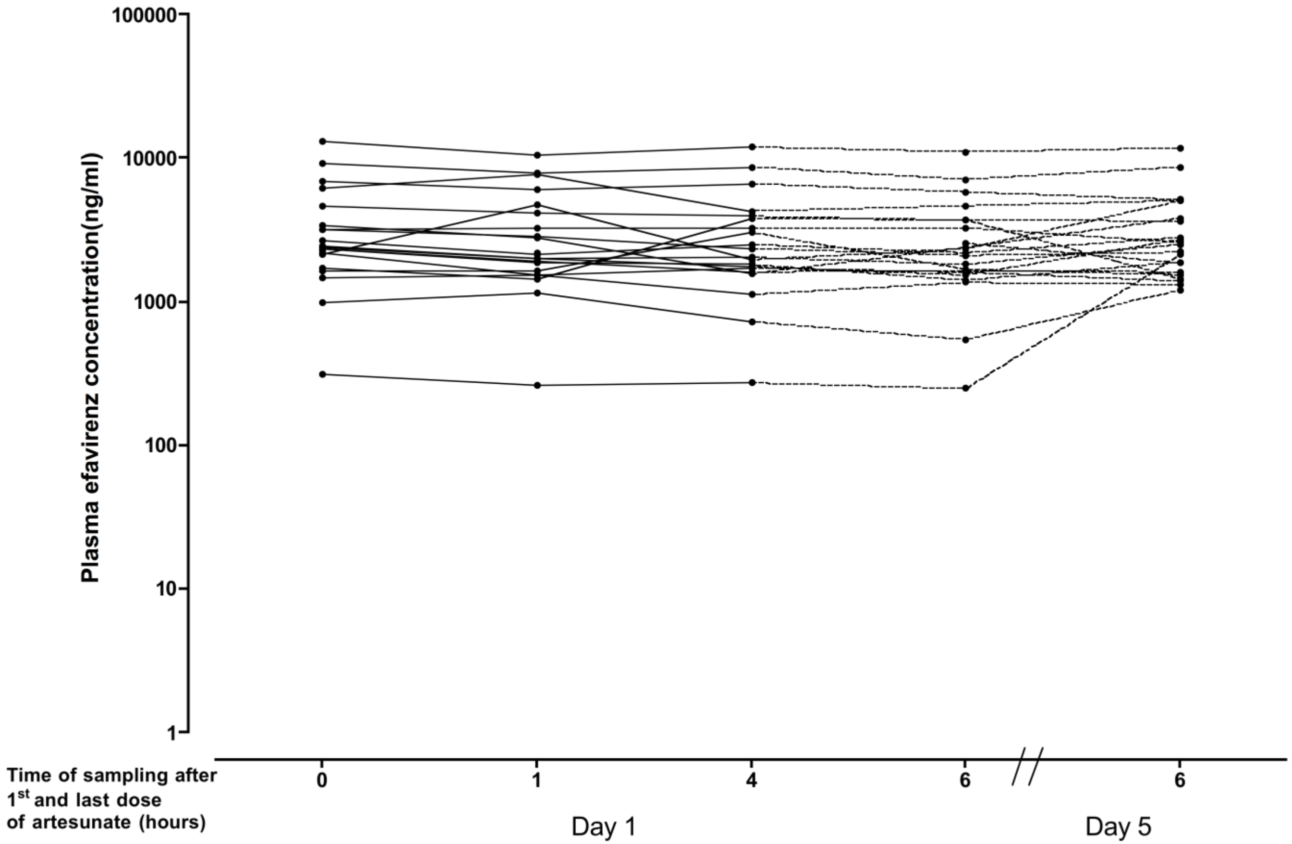


Figure 7.1. The kinetics of plasma efavirenz concentrations among Ghanaian HIV-infected patients before, during the first 6 hours after first dose of 200mg artesunate and 6 hours after the last dose of artesunate on day 5. Each dot represents efavirenz concentration at a time point and broken lines are to connect efavirenz concentrations for one patient.

Correlation between central nervous system symptomatology and mid-dose efavirenz concentrations: CNS symptoms are among the commonest manifestations of efavirenz-related toxicity. The three most common CNS symptoms at presentation were headaches, difficulty sleeping and nightmares. However, among these 3 symptoms only nightmares were present in a significantly higher proportion of HIV-infected patients on efavirenz (9/22) than controls (3/21), p=0.03. Among patients on efavirenz, the mid-dose concentration among those with complaints of nightmares was 3,184ng/ml (range of 1,732 to 13,060ng/ml) compared with 2,319ng/ml (range of 312.9 to 4,606ng/ml), p=0.025 for those without nightmares. However on day 5, there were no differences in the plasma efavirenz concentrations among those with persisting symptoms of nightmares and those whose nightmares resolved. Also no significant correlations were observed between the number of CNS symptoms and efavirenz concentrations before or after the course of artesunate therapy.

Artesunate and Dihydroartemisinin pharmacokinetics: Artesunate was not detected in any of the plasma samples tested whiles its metabolite Dihydroartemisinin (DHA) was detected at a much lower than expected concentrations in both groups of patients. These unexpected results prompted a search to determine whether the findings were due to experimental errors or to biologically plausible reasons. The strongest among the authors' suspicions were experimental errors probably due to heat-inactivation of plasma samples prior to drug assays for Artesunate and DHA. To test this hypothesis, plasma was spiked with standards of Artesunate and DHA and heated at 58^0C for 10 minutes. Heating was shown to degrade both artesunate and dihydroartemisinin as shown in Figures 7.2A and 7.2B. For instance, at a concentration of 1500μM of both Artesunate and DHA, recovery after heating was 8.7% and 8.5% respectively compared with non-heated samples. Back-up plasma samples were then shipped to Liverpool and Artesunate and DHA quantification repeated without heating these samples. Unfortunately, neither artesunate nor DHA were detected in any of the samples probably due to poor storage or sample transfer conditions.

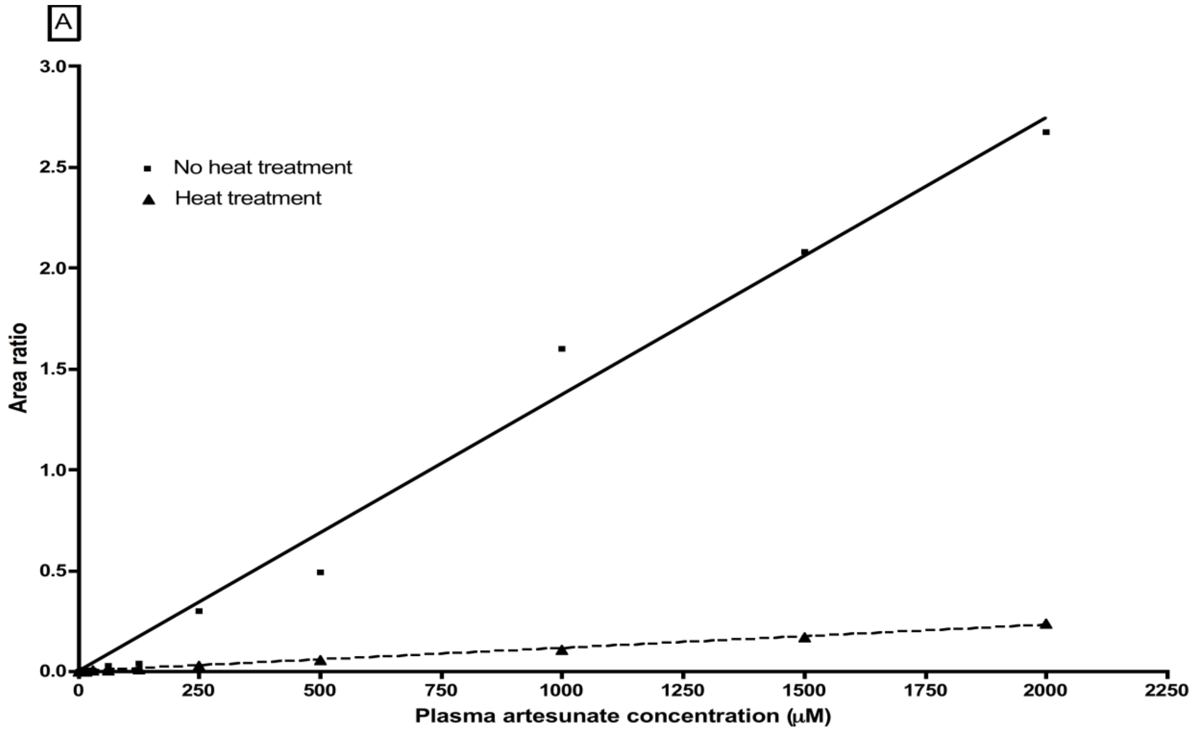


Figure 7.2A. The effect of heating plasma spiked with serial dilutions of Artesunate at 58^0C for 10 minutes in the laboratory. The solid lines represent linear regression best-fit plot for samples with no heat treatment while the broken line represent that for heat-treated samples.

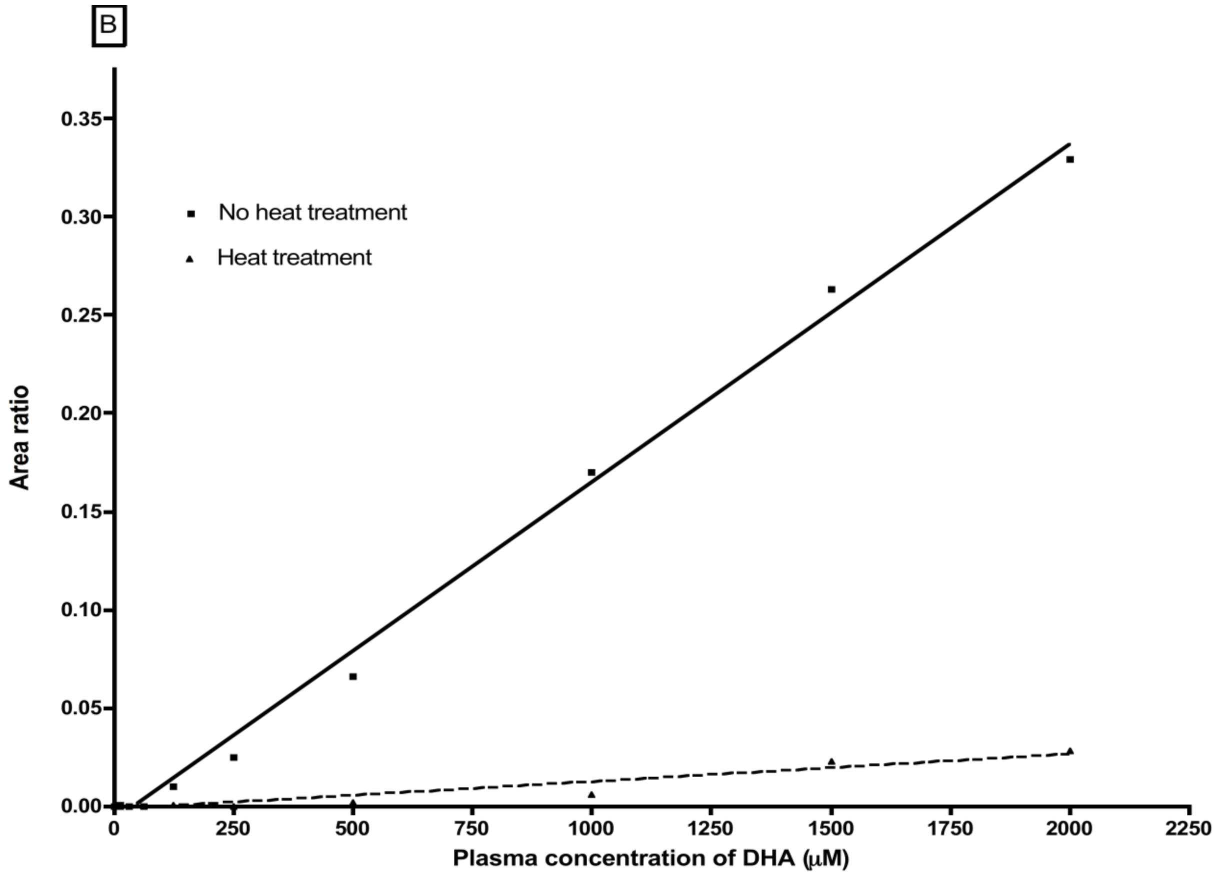


Figure 7.2B. The effect of heating plasma spiked with serial dilutions of Dihydroartemisinin (DHA) at 58^{0}C for 10 minutes in the laboratory. The solid lines represent linear regression best-fit plot for samples with no heat treatment while the broken line represent that for heat-treated samples.

In the light of these challenges, pharmacokinetic parameters were only calculated for DHA because it could be detected and quantified at a relatively appreciable concentrations and are presented below. The median time to maximum concentration of DHA in the serum among controls was 2.5 hours (range 1.0 to 4.0 hours) compared with HIV-infected patients of 4.0 (range, 0.0 to 4.0 hours), p=0.90. The median of the maximum concentration (C_{max}) of DHA among controls were significantly higher than HIV-infected patients 189.1ng/ml (range, 65.8 – 1930) vs 88.0ng/ml (0.0 – 1465), p=0.002. Also the area under the curve AUC_{0-6h} among controls was higher among controls than HIV-infected patients: 820.9 (range, 334.3 to 9535ng*hr/mL) vs 353.8 (range, 55.0 to 7776ng*hour/mL), p=0.0004.

Discussion

This study has attempted to investigate the effect of steady state efavirenz on the PK of artesunate/DHA. Primarily results from the PK of artesunate and DHA could not be reliably interpreted due to experimental challenges during analysis of plasma samples. However among the other objectives of this study, it was observed that during and after the administration of artesunate no clinically significant changes in the concentrations of efavirenz were noted. Thirdly, this study shows that there is significant resolution of symptoms and signs of clinically presumed malaria among both groups of patients after a course of artesunate without the occurrence of significant symptomatic or haemato/biochemical toxicity from the anti-malarial. Finally, efavirenz was well tolerated with no additional adverse toxicity events observed over that which was present before artesunate was initiated.

Following the administration of oral artesunate, it is rapidly absorbed within 15 minutes, and undergoes extensive first pass metabolism to DHA[540]. From studies that have defined both artesunate and DHA PK following oral artesunate intake, DHA C_{max} and AUC have exceeded those of artesunate with some studies reporting more than a 10-fold increase in DHA AUC over that of artesunate[541-551]. Thus orally administered artesunate is considered a pro-drug of DHA from PK, bioavailability and bioequivalence data. These findings could explain why DHA could still be detected in samples after being heated compared with artesunate although both compounds underwent thermal degradation to similar extents (Figures 7.2A and 7.2B). It is hoped that in future studies the observed trend of a lower $AUC_{0\text{-}6h}$ and C_{max} for DHA among

HIV-infected patients compared with control patients would be ascertained and if confirmed, its clinical impact assessed.

During and after the course of artesunate, the steady state concentrations of efavirenz were maintained within the therapeutic range of 1,000ng/ml to 4,000ng/ml for the significant proportion of patients (n=16) and for patients who were exposed to supra-therapeutic concentrations (n=5). One patient had sub-therapeutic exposure of efavirenz on day 1 due to suspected non-adherence because on day 5, efavirenz drug levels were back to within normal levels as shown in Figure 7.1. The reductions in efavirenz steady-state concentrations observed during and after the administration of artesunate could possibly be of minor clinical relevance or could be due to clearance of efavirenz towards it C_{min} since all patients reported taking their dose at night and sampling was performed within 12 to 18 hours post-dose.

It is well known that high plasma concentrations of efavirenz are associated with the development of central nervous system toxicity[122]. Given the prospective design of this study, we took advantage to evaluate patients for symptoms of efavirenz toxicity before and after taking artesunate. Most of the CNS symptoms reported by patients on efavirenz were of recent onset (median of 1 week), had coincided with the symptoms of presumed malaria, were not distinguishable from those of control patients at baseline except for nightmares and tended to resolve upon treatment of malaria. The significant difference in the median concentration of efavirenz among patients with nightmares on day 1 was not found among those whose nightmares persisted after treatment of malaria. Also among patients with supra-therapeutic exposure (n=5) to efavirenz, CNS symptoms had resolved in all on day 5.These findings indicate that it is probably less likely that efavirenz concentrations predict CNS toxicity with specificity among patients with symptoms of malaria. It should also be remembered that these patients had been on efavirenz for a median of 26.5 months and therefore are expected to have developed tolerance for these adverse events. The fact that no symptomatic malaria treatment failure was observed on artesunate among HIV-infected patients taking long-term efavirenz and that no occurrence or worsening of toxicity of either medications were witnessed provides some reassurance for their use hopefully with other long-acting antimalarials such as lumefantrine in accordance with WHO recommendations in the Ghanaian population.

There are several limitations to this study worth noting. Sampling for artesunate/DHA PK measurements were not as intensive as it should be because initial attempts to sample using a schedule of 0, 15, 30, 60, and 90 minutes followed by further sampling at 2, 4, 8 and 12 hours were refused by all the initial controls and HIV-infected patients who were approached most of whom were not willing to spend that length of time in the hospital. Thus a compromise in the sampling schedule was effected which affected the ability to study the full pharmacokinetic profile of artesunate or DHA as it well-known that a relative lack of sampling points in the early post-dose period can result in much of the subjects' artesunate exposure being missed[552]. It is unfortunate that plasma samples were heated prior to measurement of artesunate and DHA concentrations and this undoubtedly has significantly affected the interpretation and applicability of the PK results of this study. Heating the samples prior to measurement of artesunate/DHA was a laboratory requirement for processing these samples. However, similar or even worse results were obtained from back-up samples shipped later from Ghana. These findings probably reflect the instability of both artemisinin compounds[553]. The validated liquid chromatography tandem mass spectrometry employed in this study for the detection and quantification of artesunate and DHA is considered the gold standard for the analysis of these drugs in biological matrices[554, 555] compared with other modalities such as the on-line post-column alkali derivatisation with UV detection[556], electrochemical detection[557] and chemiluminescent detection[558]. Thus it is a reasonable possibility that the detection and quantification method employed could not have been the reason for the low concentrations of DHA detected.

Again, it is uncertain from the results of efavirenz steady concentrations whether the reductions in concentrations observed during the co-administration of artesunate were due to efavirenz ebbing towards its C_{min} or were from pharmacokinetic effects of artesunate. The steady state kinetics of efavirenz among HIV-infected patients not on artesunate anti-malarial therapy for comparison could prove useful in providing some idea on whether the anti-malarial causes clinically relevant reductions in the concentrations of efavirenz. However, it should be noted that these reductions were within the therapeutic range of efavirenz and probably of minor clinical relevance given that that interassay variability of efavirenz quantification is within 10%. Indeed several valuable lessons have been learnt by the author from the challenges in this endeavour that should prepare him for these future studies aimed at answering these important scientific questions.

In conclusion this study has shown that there are no significant changes in the steady state plasma concentration of efavirenz with co-administration of artesunate for the treatment of malaria. Overall, artesunate was well tolerated by patients on efavirenz and appeared clinically effective in relieving symptoms and parasitemia with no adverse toxicity events from efavirenz. This data is reassuring and future studies are needed to corroborate these findings.

CHAPTER EIGHT

The impact of selected CYP2B6, CYP2A6, UGT2B7 and CAR single nucleotide polymorphisms on plasma steady state concentrations of efavirenz, risk for neuropsychiatric toxicity and immunological outcomes in Ghanaian HIV-infected patients.

8.0 Introduction

Following standard doses of antiretroviral drugs, huge inter-individual variability has been observed, up to (CV%) of 75->110% occurs for NNRTIs and PIs, from prospective clinical trials[289]. The causes of this variability are multifactorial and include poor adherence, body weight, gender and interacting medications[559, 560]. Host genetic polymorphisms may account for some of the variation in pharmacokinetics and responses to ART. Genetic variability in the drug metabolising enzymes (e.g. cytochrome P450, glucoronyl transferase) or drug transporters (e.g. MDR1, MRP1 & 2), prevalent at differing frequencies across ethnic groups probably explain some of the differences between populations.

Efavirenz is an essential component of the preferred non-nucleoside reverse transcriptase regimen for the initial treatment of HIV-1 infection in both the industrialised[111] and developing[1] countries. Despite the proven potency and favourable tolerability of efavirenz-based regimen, large inter-individual variability in plasma efavirenz concentrations predisposes to the development of treatment- limiting toxicity or failure to achieve durable viral load suppression[122, 561]. Efavirenz is administered orally as a single fixed dose of 600mg in adults and undergoes phase I oxidative metabolism primarily by the hepatic CYP2B6 enzyme[276] with minor contributions from

CYP3A4 and the recently identified CYP2A6. Subsequent phase II metabolism involves glucuronidation of oxidised efavirenz metabolites by the UGT2B7 enzyme. Genetic variations in the enzymes responsible for the metabolism of efavirenz and nuclear factors such as the constitutive androstane receptor (CAR) involved in the induction of enzyme expression may partially explain the inter-individual variability in plasma efavirenz concentrations.

It has become apparent that the profound inter-individual differences in hepatic CYP2B6 expression and enzymatic activities may result in variable systemic exposure and therapeutic response to the drugs metabolized by CYP2B6. Indeed considerable polymorphisms exist for cytochrome P450 2B6[280], the major enzyme for detoxification of the NNRTIs EFV and NVP. An allelic variant (G516T) which is more common in African Americans (TT 20%) than Hispanics (6.7%) or Caucasians (3.4%) was associated with slower clearance of EFV leading to a hierarchy of EFV exposure (and associated CNS toxicity) in the rank order: African Americans > Hispanics > Caucasians[209]. Whilst considerable work has been done showing the effect of the G516T mutation on efavirenz levels, the effect of the T983C mutation in the CYP2B6 isoform on either efavirenz or nevirapine levels have emerged in recent times and the minor allele of this gene is relatively common among Ghanaians[35, 36]. In addition to polymorphisms in CYP2B6, this isoenzyme is highly inducible and chemically mediated induction is regulated at the transcriptional level through complex interactions of nuclear factors such as CAR and PXR. In-vitro evidence suggests that efavirenz is one of the selective agents for human CAR mediated CYP2B6 induction but to the best of my knowledge no in-vivo studies have been conducted to assess the contribution of polymorphisms in the CAR C>Trs2307424 receptors on efavirenz exposure in an HIV population in sub-Saharan Africa. Furthermore it has been postulated that in the presence of aberrant 2B6 expression and/or function, CYP P450 2A6 may assume an essential role in hydroxylation of efavirenz via the 7-OH accessory pathway hence polymorphisms in isoforms of this enzyme in the populations where there is a high prevalence of variants of 2B6 assumes importance[281].

The aims of this study were first to investigate the frequencies of the 516G>T and 983T>C Single Nucleotide Polymorphisms (SNPs) of the CYP2B6, the allelic variants of CYP2A6*9B, the 802C>T and 735A>G SNPs of the UGT2B7 and the C>T (rs2307424) SNP of CAR in a cohort of Ghanaian HIV patients. The second objective was to assess the impact of the genetic polymorphisms in the CYP2B6, CYP2A6, UGT and CAR on the plasma concentrations of efavirenz as well as possible gene-gene interactions. Finally, the pharmacodynamic impact of the selected SNPs and efavirenz exposure on the risks for CNS toxicity and long-term treatment outcomes such as immunological failure were retrospectively assessed in a subset of patients.

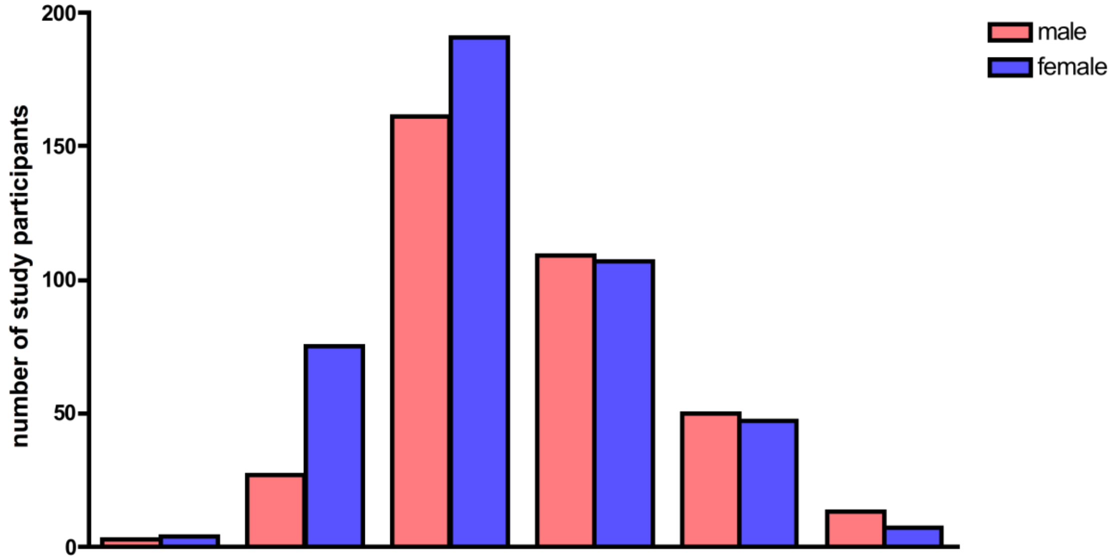
8.1 Methods

Please refer to chapter two from subsections 2.8.1.1 to 2.8.1.5.

8.2 Results

Demographic and anthropometric data: The median (range) age of study participants was 40 (17 – 68) years, with a female to male ratio of 2:1. The age and gender distribution of patients involved in the study are shown in Figure 8.1. The median (IQR) body mass index of males of 22.7 (20.0 – 26.1 kg/m^2) was not significantly higher than 22.1 (20.1 – 26.1 kg/m^2) in females, p=0.10. Five hundred and seventy-eight 578

(72.3%) patients were on cART while 222(27.7%) were naïve at time of sampling. Of those on cART, 521 were on efavirenz-based therapy, 56 were on nevirapine-based therapy while 1 was on nelfinavir-based therapy; 277 (47.8%) were on zidovudine plus lamivudine, 300 (52.2%) on stavudine plus lamivudine and 1 on didanosine plus lamivudine nucleoside backbone.



Age range	<20	21-30	31-40	41-50	51-60	61-70
male	3	27	161	109	50	13
female	4	75	191	107	47	7

Figure 8.1. Age and gender distribution of study participants who were involved in the pharmacogenomic study.

Frequencies of genetic polymorphisms in efavirenz metabolising enzymes: Of the 800 samples from which genomic DNA were extracted, genotyping was successful for 705 (88%) of CYP2B6 G516T, 701 (88%) of CYP2B6 T983C, 678 (85%) of CYP2A6*9B, 704 (88%) of UGT2B7*1A, 697 (87%) of UGT2B7*2 and 695 (87%) of CAR C>Trs2307424 SNPs respectively (see Figure 8.2 for study profile). When a Chi-square test of observed versus predicted genotype frequencies was conducted, all polymorphisms were found to be in Hardy Weinberg equilibrium. Allele frequency and genotypes of the SNPs analysed in this study is shown in Table 8.1. Briefly, the minor allele frequencies for CYP2B6 G516T and T983C SNPs were 0.48 and 0.04 respectively; that for UGT2B7 _735 and _802 were 0.15 and 0.23 respectively and for CYP2A6*9B and CAR C>T rs2307424 were 0.03 and 0.07 respectively. Overall, the genotype frequencies of CYP2B6 G516T were GG 208 (29.5%), GT 320 (45.4%), TT 177(25.1%); CYP2B6 T983C were TT 639 (91.2%), CT 61 (8.7%), CC 1 (0.1%); UGT2B7_735 A>G were AA 507(72.0%), AG 172 (24.4%), GG 25 (3.6%); UGT2B7_802 were CC 391 (56.1%), CT 287 (41.2%), TT 19 (2.7%); CYP2A6*9B were CC 635 (93.7%), CA 42(6.2%), AA 1(0.1%) and CAR C>Trs2307424 were CC 602(86.6%), CT 89(12.8%) and TT 4(0.6%).

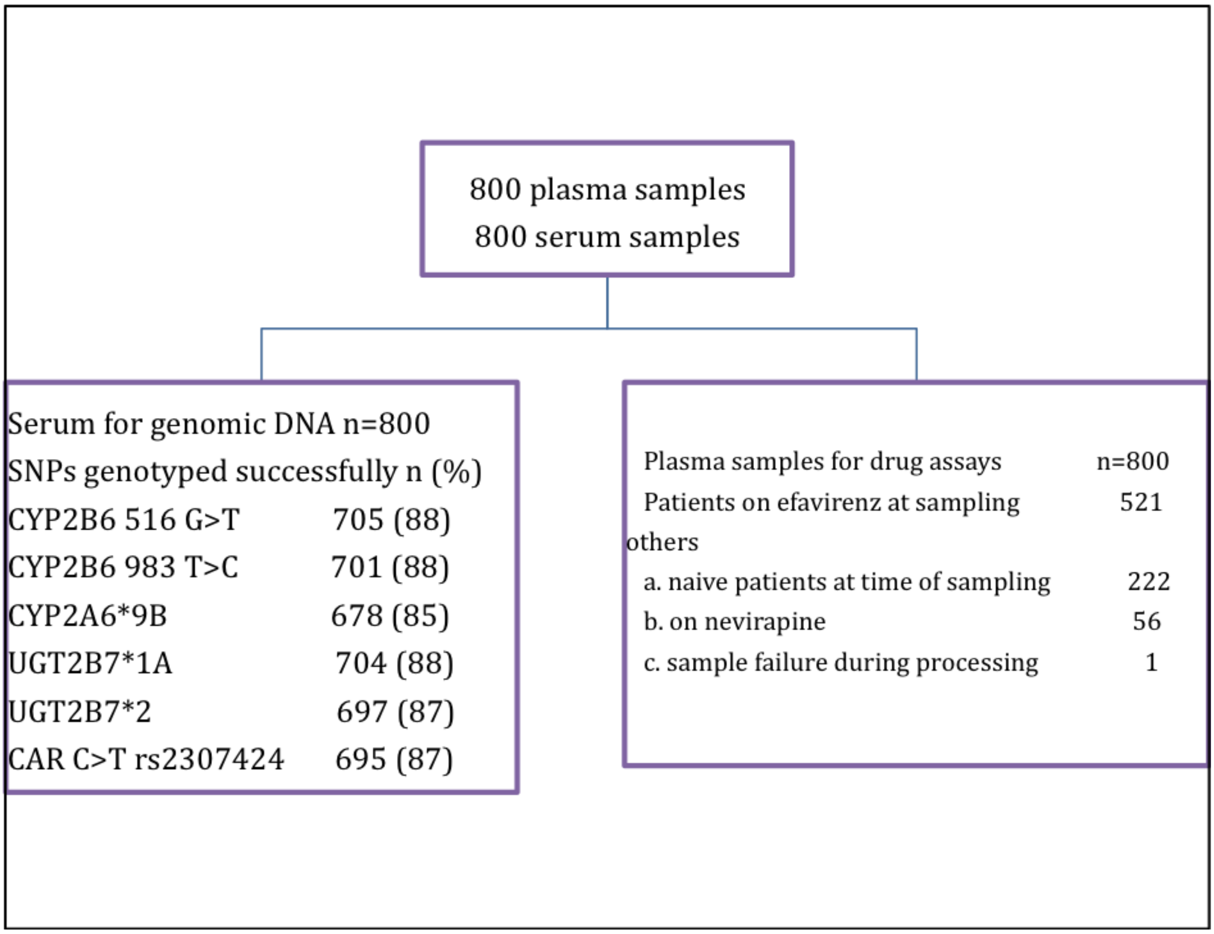


Figure 8.2. The profile of the pharmacogenomic study.

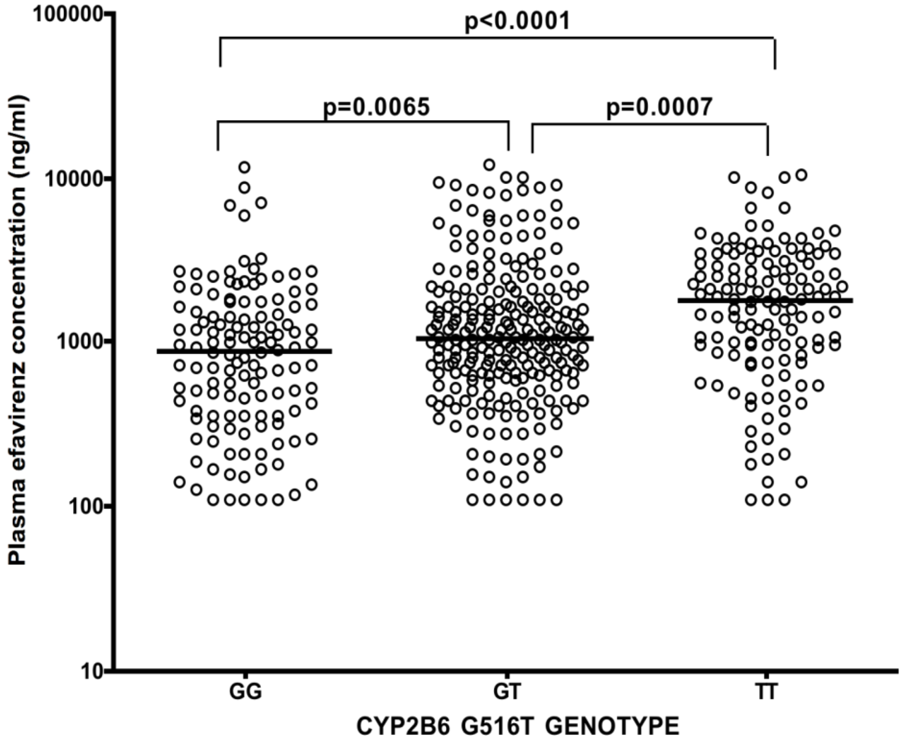
Table 8.1 The genotype and allele frequencies of selected SNPs of enzymes involved in metabolism of efavirenz.

SNP	Genotype			Allele n (%)	
CYP2B6 516G>T	GG 208	GT 320	TT 177	G 730(52)	T 674(48)
CYP2B6 983T>C	TT 639	TC 61	CC 1	T 1339 (96)	C 63 (4)
CYP2A6*9b	CC 635	CA 42	AA 1	C 1312 (97)	A 44(3)
UGT2B7*1A	AA 507	AG 172	GG 25	A 1186 (85)	G 222 (15)
UGT2B7*2	CC 391	CT 287	TT 19	C 1069 (77)	T 325 (23)
CAR C>T (rs2307424)	CC 602	CT 89	TT 4	C 1293 (93)	T 97 (7)

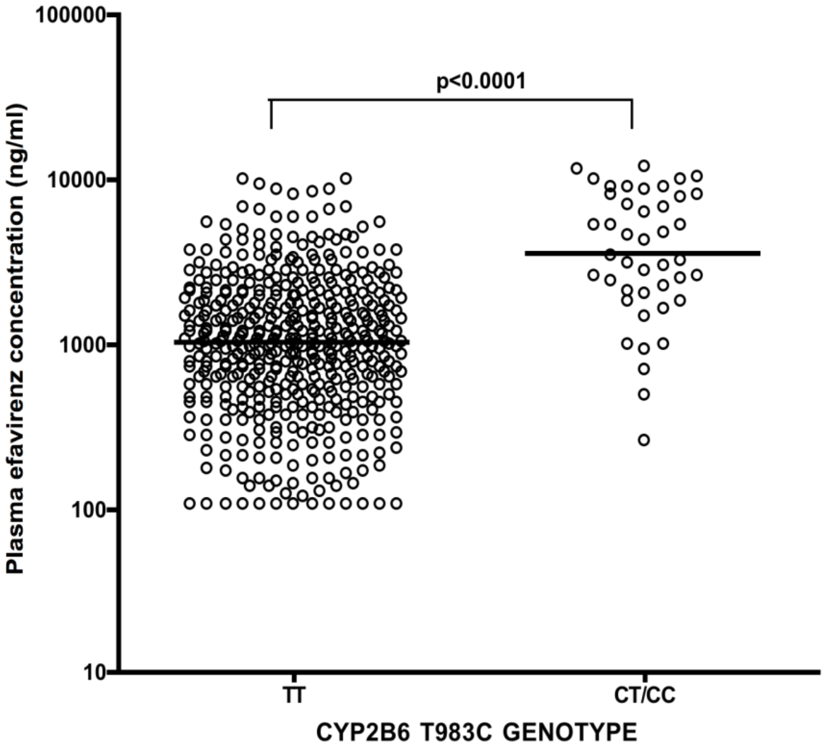
Mid-dose plasma efavirenz concentrations: 521 patients on efavirenz containing cART had mid-dose plasma efavirenz determined. Median (IQR) concentration of plasma efavirenz was not significantly different in males 1090 (533.3 – 2173 ng/ml) compared with females 1083 (559.9 – 2102 ng/ml). 46% had sub-therapeutic mid-dose concentrations of plasma efavirenz (<1000ng/ml), 44% were within the therapeutic range (1000 – 4000 ng/ml) and 10% had supra-therapeutic concentrations of efavirenz.

Selected SNPs and impact on plasma efavirenz concentrations: Concentration of efavirenz was significantly higher in individuals homozygous for the variant allele (TT) at position 516 of the CYP2B6 gene (TT [n=128]: 1800ng/ml vs 1073ng/ml and 929 ng/ml for GT [n=226] and GG [n=120] individuals respectively; p<0.0001). Similarly, concentration of efavirenz was significantly higher in individuals homozygous or heterozygous for the variant allele (CC) at position 983 of the CYP2B6 gene [CC n=1 and TC n=42]: 3235ng/ml vs 1053 ng/ml for TT [n=429] individuals; p<0.0001). Also the concentration of efavirenz was significantly higher in individuals homozygous or heterozygous for the variant allele (AA) at position 1836 of the CYP2A6 gene [AA n=1 and CA n=27]: 2192 vs 1093 ng/ml for CC [n=425] individuals; p<0.001). However the concentration of efavirenz was not significantly different in individuals homozygous or heterozygous for the variant allele (GG) at position 735 of the UGT2B7 gene [GG n=13 and AG n=112]: 802.9ng/ml vs 1160ng/ml and for normal AA [n=345] individuals of 1107ng/ml (p=0.84). Also concentration of efavirenz was not significantly different in individuals homozygous or heterozygous for the variant allele (TT) at position 802 of the UGT2B7 gene [TT n=16 and CT n=192]: 823.3 ng/ml vs 1034ng/ml and for normal CC [n=258]- 1172ng/ml individuals, p=0.67. Finally the concentration of efavirenz of 1001 ng/ml was not significantly different in individuals homozygous for the variant allele (TT) n=1 or heterozygous variant allele [CT] n=66 and for normal CC [n=395] individuals 1130ng/ml, p=0.3 of the CAR C>T rs2307424 gene. The impact of each selected SNP on the mid-dose plasma concentration of efavirenz is depicted in Figures 8.3A-F.

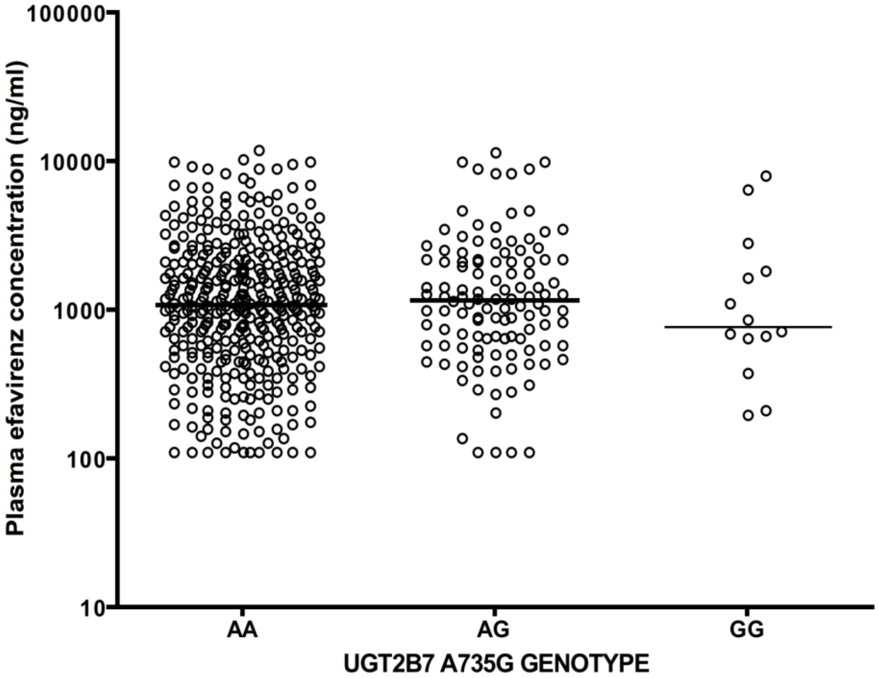
3A


p<0.0001
p=0.0065
p=0.0007
100000
10000
1000
100
10
Plasma efavirenz concentration (ng/ml)
GG
GT
TT
CYP2B6 G516T GENOTYPE

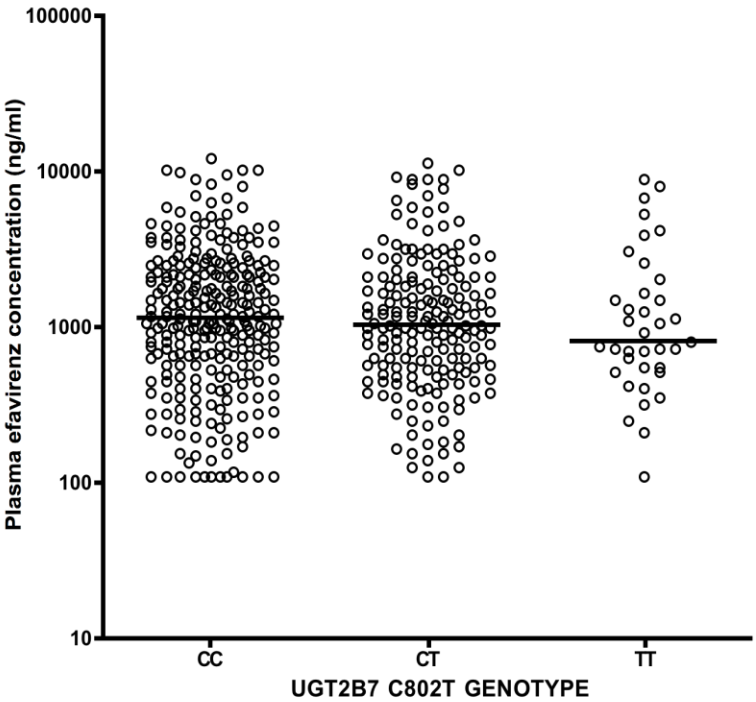
3B


p<0.0001
100000
10000
1000
100
10
Plasma efavirenz concentration (ng/ml)
TT
CT/CC
CYP2B6 T983C GENOTYPE

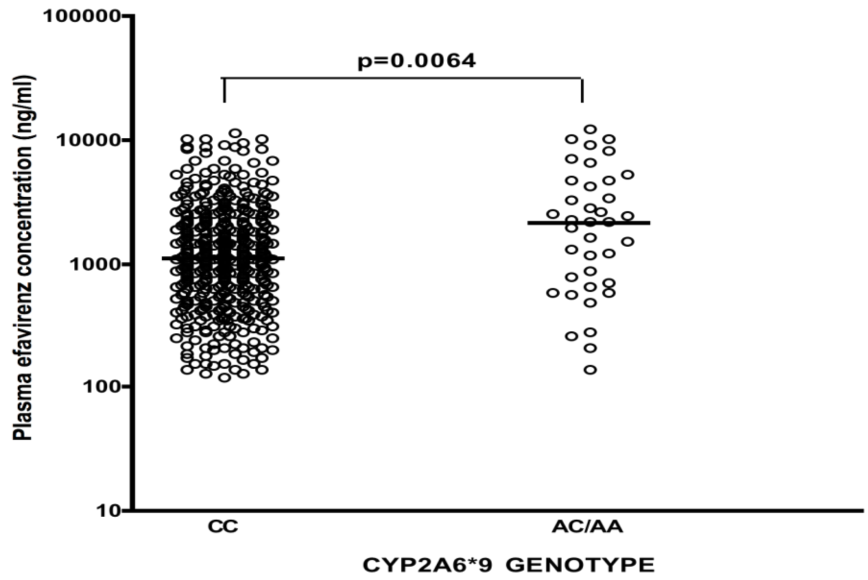
3C


100000
10000
1000
100
10
Plasma efavirenz concentration (ng/ml)
AA
AG
GG
UGT2B7 A735G GENOTYPE

3D


100000
10000
1000
100
10
Plasma efavirenz concentration (ng/ml)
CC
CT
TT
UGT2B7 C802T GENOTYPE

3E



3F

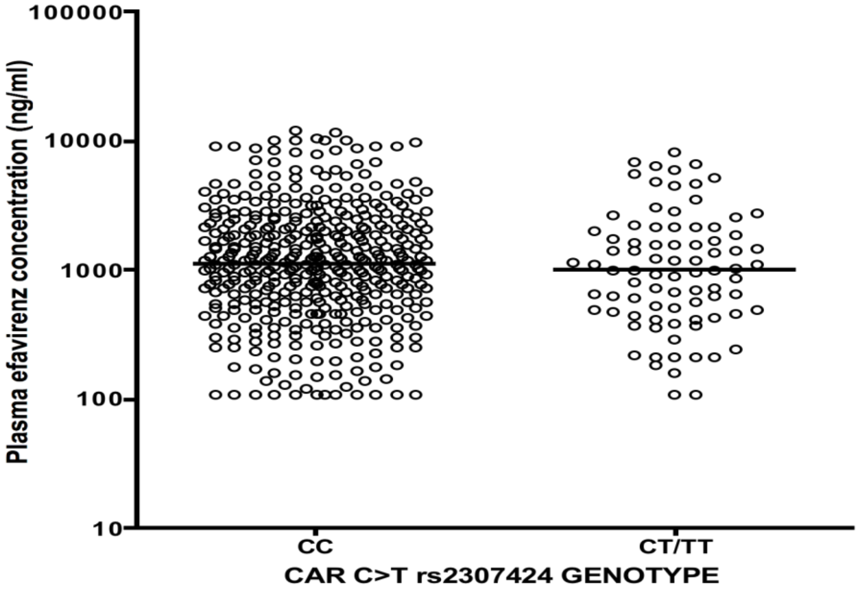


Figure 8. 3A-F: The impact of selected SNPs on mid-dose plasma efavirenz exposure. 3A shows the impact of polymorphism of CYP2B6 516G>T; 3B shows the impact of polymorphisms of CYP2B6 983T>C; 3C shows the impact of polymorphisms of UGT2B7 735 A>G; 3D shows the impact of polymorphisms of UGT2B7 802 C>T; 3E shows the impact of polymorphisms of CYP2A6 1836 C>A; and 3F shows the impact of CAR C>Trs2307424 on the steady state concentrations of efavirenz. Each circle represents concentration of efavirenz for one study participant and each horizontal line represents a median.

Gene-gene interactions: It is well known that polymorphisms in CYP2B6 account for a significant proportion of genetically mediated inter-individual variation in efavirenz exposure. A 2-way ANOVA analysis was conducted to assess the impact of variants of CYP2A6*9B, CAR C>Trs2307424, UGT2B7*1A and UGT2B7*2 on efavirenz exposure by controlling for CYP2B6 516G>T and 983T>C individually, including evaluation for possible gene-gene interactions. This analysis was performed on 420 patients who had a full set of genotypes successfully performed and efavirenz measurements. Significant interaction was observed between variants of CYP2B6 516G>T and CYP2B6 983T>C with a p-value of 0.0006 for interaction between these two SNPs. Figure 8.4A shows that, as the number of mutants in the two CYP2B6 SNPs increases the plasma concentrations of efavirenz significantly increases accordingly with median (IQR) concentrations of 728 (311 – 1293 ng/ml), 1019 (613 – 1788 ng/ml), 1973 (968 – 3534 ng/ml) and 5854 (2083 – 9289 ng/ml) with nil, 1, 2 and 3 mutations respectively corresponding to 1.4-fold, 2.7-fold and 8.0-fold increases compared with no mutations in the CYP2B6 516/983 composite variants.

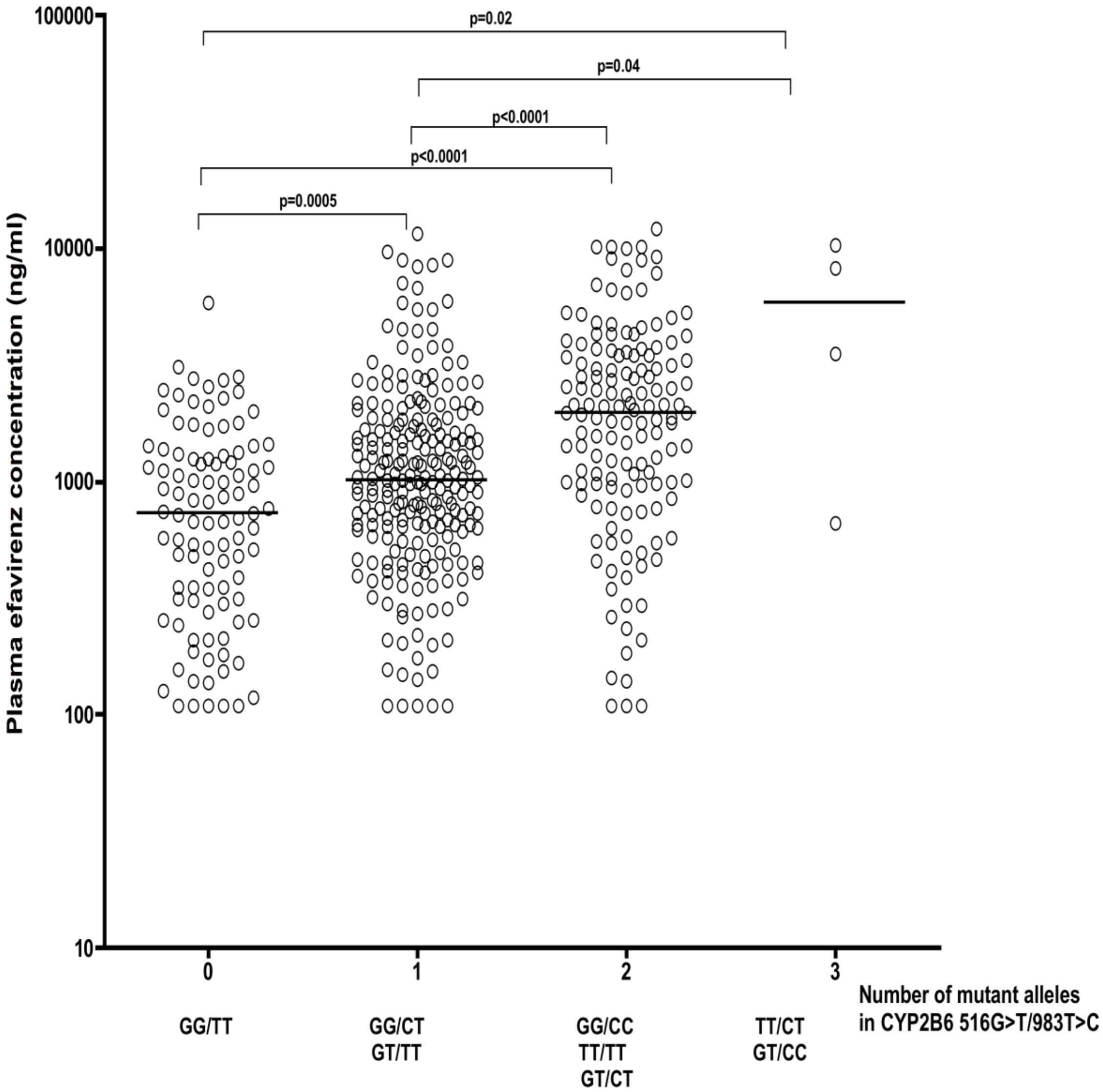


Figure 8.4A. The influence of cumulative mutations in the CYP2B6 516G>T or 983T>C polymorphisms on plasma efavirenz mid-dose concentrations.

Next, the impact of gene-gene interaction between CYP2A6*9B and the CYP2B6 G516T and T983C SNPs were performed and as shown in Figure 8.4B, the slow metaboliser variant of the CYP2A6*9B was independently associated with higher efavirenz exposure after adjusting for CYP2B6 516G>T, p=0.0006 for CYP2A6*9B effect on efavirenz variance; p=0.45 for interaction. In a posthoc pairwise analysis using Mann-Whitney's U-test, it emerged that heterozygotes of 516G>T with mutant variants of 2A6*9B compared with wild type variants had a significantly higher efavirenz exposure. However after adjusting for CYP2B6 983T>C variants, CYP2A6*9B variants did not exert a significant independent effect on efavirenz variance (Figure 8.4C), although no significant interactions were observed between the two genes. Overall, the CYP2A6*9B variants appear to exert an impact towards higher efavirenz exposure in the presence of increasing numbers of SNPs in the two CYP2B6 alleles (Figure 8.4D).

Generally the selected SNPs in UGT2B7*1A, UGT2B7*2 and CAR rs2307424 did not interact significantly with either CYP2B6 516G>T or T983C SNPs individually or as a composite in explaining the impact exerted by these SNPs on the variance of plasma efavirenz exposure (Table 8.2). However, there was a trend towards lower exposure to efavirenz among patients with the CAR C>T rs2307424 mutant SNP in the absence of mutant SNPs in the CYP2B6 alleles as summarised in Figure 8.4E. Conversely among patients with CYP2A6*9B SNPs, there was a trend towards higher plasma efavirenz exposure in the presence of loss-of-function SNPs in the CYP2B6 alleles (Figure 8.4E).

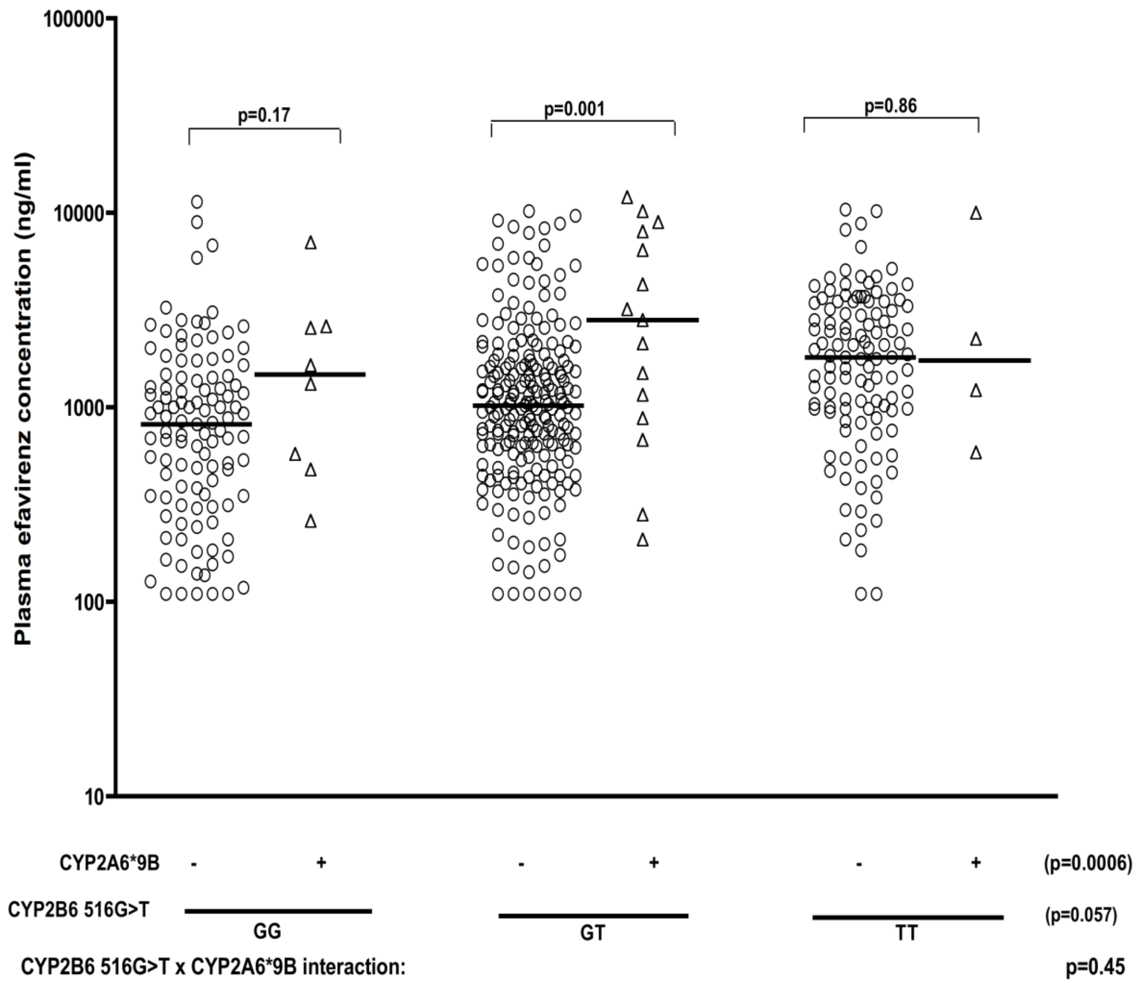


Figure 8.4B. Influence of CYP2A6*9B slow metaboliser genotype and CYP2B6 516G>T on plasma efavirenz mid-dose concentrations.

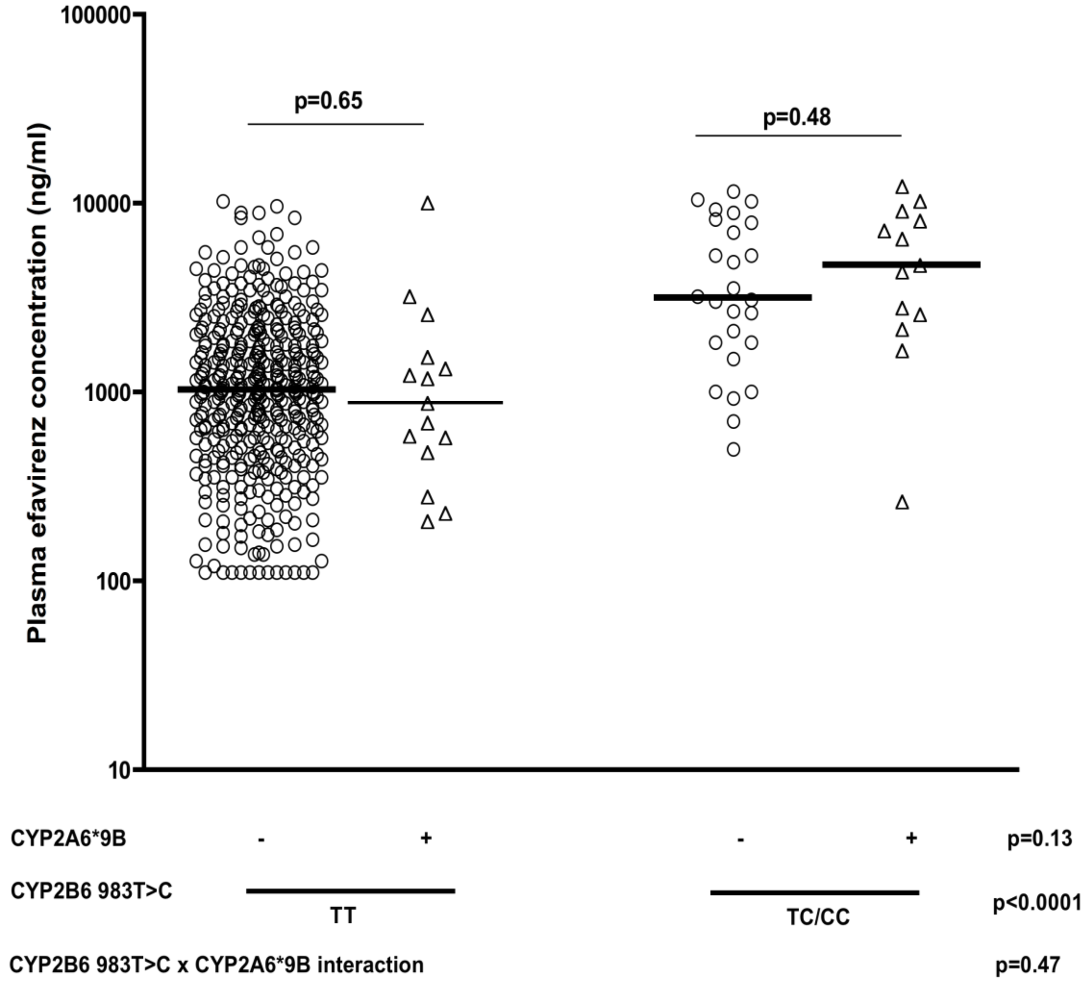


Figure 8.4C. Influence of CYP2A6*9B slow metaboliser genotype and CYP2B6 983T>C on plasma efavirenz mid-dose concentrations.

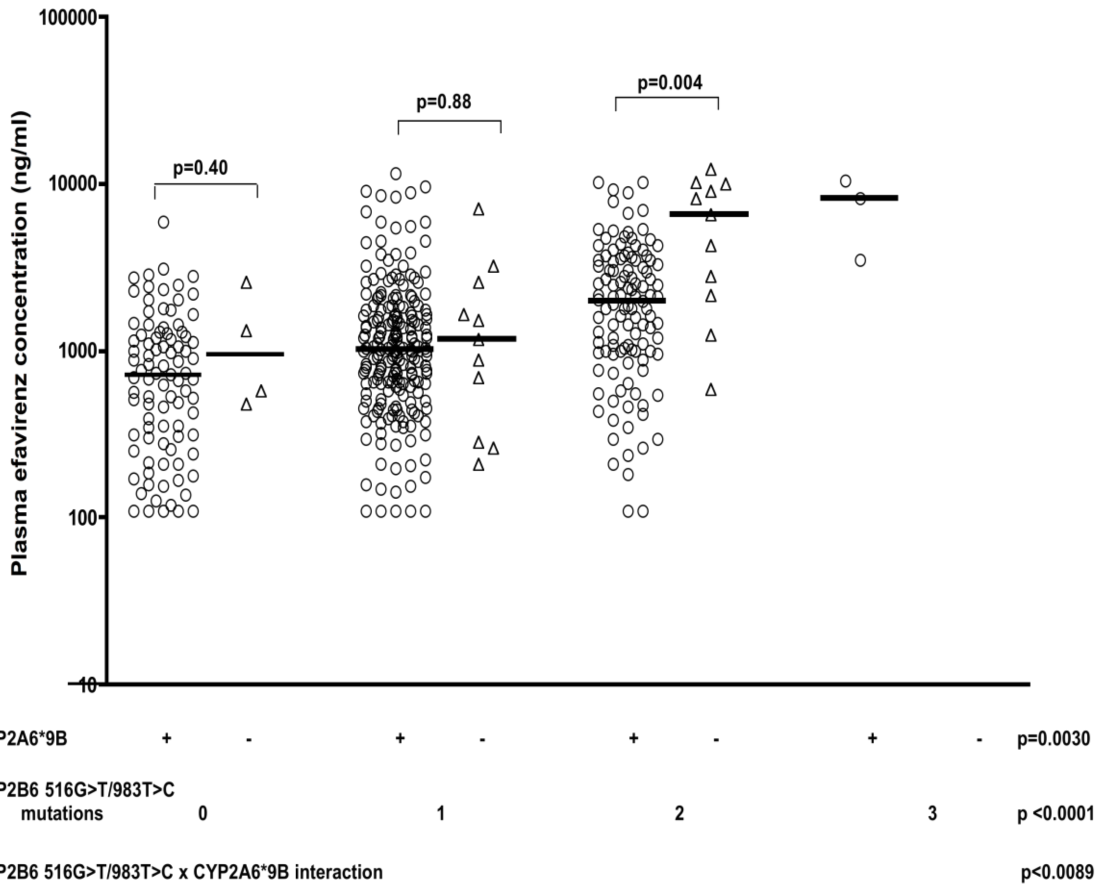


Figure 8.4D. The influence of CYP2A6*9B slow metaboliser genotype on cumulative mutations in the CYP2B6 516G>T or 983T>C polymorphisms on plasma efavirenz mid-dose concentrations. There were significant interactions between the CYP2B6 composite SNPs and the CYP2A6*9B SNP.

Table 8.2 showing a post-hoc two-way analysis of variance testing for interactions between wild type and mutant type alleles for selected SNPs and a composite of CYP2B6 mutation status.

Mean ± SEM of mid-dose plasma efavirenz concentration	CYP 2B6 no mutation	CYP 2B6 1 mutation	CYP 2B6 ≥2 mutations	% total variation due to 2B6 variants (p-value)	% total variation due to variants of SNPs on each row (p-value)	% total variation due to interaction (p-value)
CYP 2A6*9B				7.23 (<0.0001)	1.91 (0.0030)	2.05 (0.0089)
No mutation	978 ± 98.3 (n=87)	1564 ± 126.7 (n=197)	2600 ± 212.7 (n=113)			
≥1 mutations	1486 ± 580.9 (n=3)	1809 ± 673.0 (n =10)	6049 ± 1353 (n =10)			
UGT2B7_705				11.86 (<0.0001)	0.07 (0.6)	0.28 (0.5)
No mutation	1000 ± 124.9 (n=64)	1600 ± 138.3 (n=149)	2758 ± 269.8 (n=93)			
≥1 mutations	981 ± 137.0 (n=26)	1516 ± 268.9 (n=58)	3260 ± 500.8 (n=30)			
UGT2B7_802				12.95 (<0.0001)	0.41 (0.2)	0.17 (0.7)
No mutation	1160 ± 137.6 (n=57)	1589 ± 151.3 (n=117)	3072 ± 361.4 (n=62)			
≥1 mutations	709.3 ± 99.5 (n=33)	1559 ± 209.0 (n=90)	2686 ± 308.3 (n=61)			
CAR C>T rs2307424				5.51 (<0.0001)	0.25 (0.3)	0.27 (0.5)
No mutation	975 ± 89.3 (n=78)	1663 ± 144.4 (n=173)	2957 ± 267.6 (n=105)			
≥1 mutations	1122 ± 452.9 (n=12)	1136 ± 169.2 (n=34)	2435 ± 444.1 (n=18)			

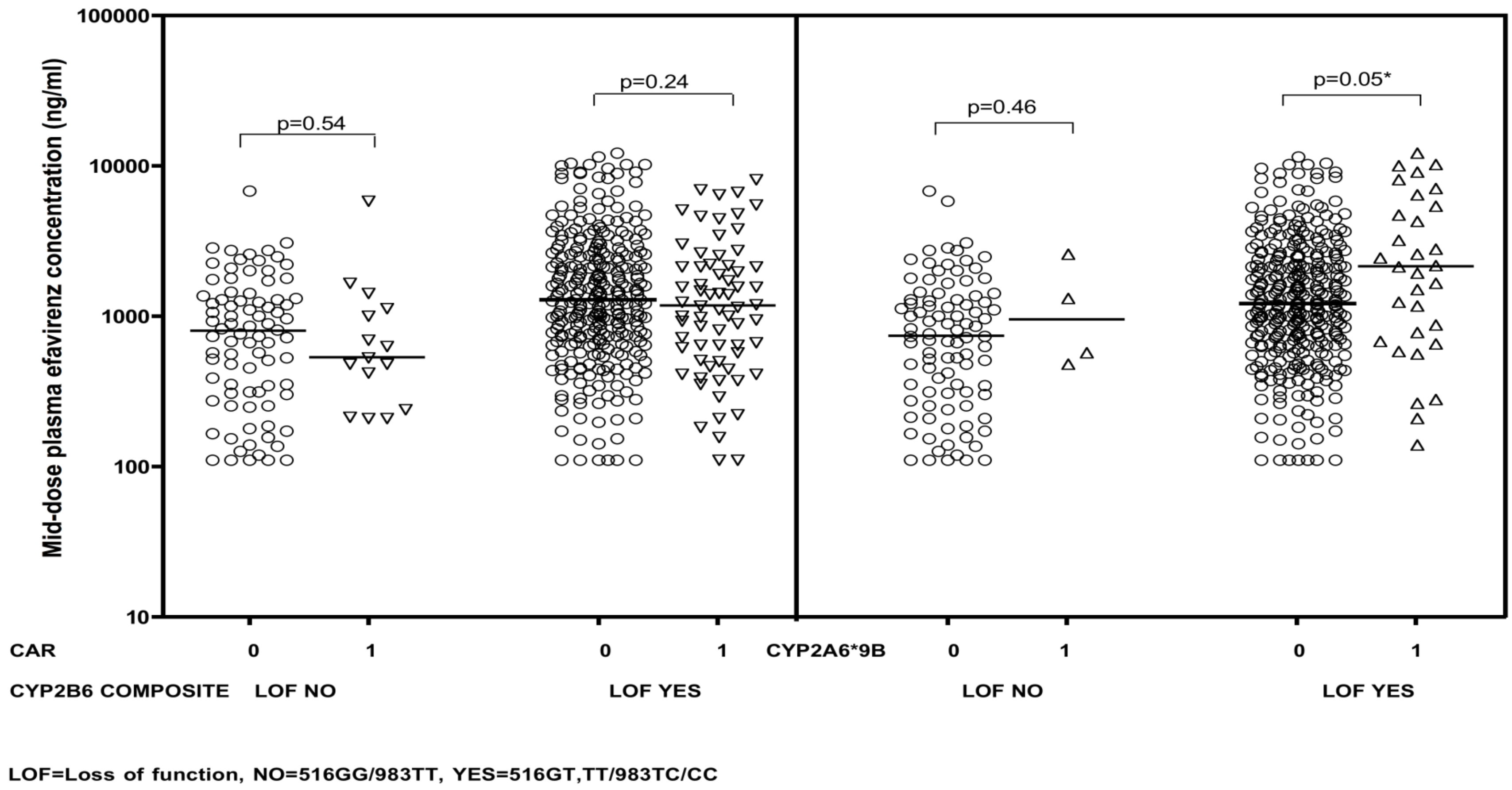


Figure 8.4E. The impact of SNPs in CARrs2307424 and CYP2A6 in relation to loss-of-function SNPs in CYP2B6 on efavirenz exposure.

Multivariate linear regression analyses: The median (range) plasma concentration of efavirenz was 1087 (110.0 – 12,146.0 ng/ml) and a mean ± standard deviation of 1792 ± 2024ng/ml with a coefficient of variation of 113%. On univariate analyses polymorphisms in CYP2B6 G516T ($p=2.5 \times 10^{-7}$); T983C ($p=4.5 \times 10^{-14}$); CYP2A6*9b p=0.002), and body weight in kilograms (p=0.008) were all significantly correlated with log transformed plasma efavirenz concentration. On multivariate analysis CYP2B6*516G>T, *983T>C and body weight were identified as independent variables of efavirenz exposure as shown on Table 8.3. The G516T and T983C SNPs in the CYP2B6 gene explained 32% and 38% respectively of the variation in mid-dose concentrations of efavirenz among this Ghanaian cohort. CAR rs2307424 polymorphism was marginally significant while increasing weight was inversely correlated with efavirenz exposure.

Table 8.3. Univariate and multivariate analyses of independent variables on the log concentration of efavirenz as dependent variable.

Covariate	Univariate probability	Multivariate probability	Multivariate coefficient (b)
CYP2B6 516G>T	2.5×10^{-7}	1.4×10^{-11}	0.32
CYP2B6 983T>C	4.5×10^{-14}	1.3×10^{-15}	0.38
CYP2A6*9B	0.002	0.60	-
UGT2B7*1	0.84	-	-
UGT2B7*2	0.24	-	-
CAR rs2307424	0.12	0.07	0.08
Age	0.23	-	-
Gender	0.84	-	-
Weight	0.008	0.016	-0.11
Height	0.45	-	-

The pharmacodynamic impact of SNPs and efavirenz exposure on clinically relevant outcomes of efavirenz-based cART: The analyses in this section are post hoc and retrospective in nature and are an attempt to evaluate associations between the pharmacogenomic and efavirenz exposure data above presented and clinically relevant outcomes presented in other chapters of this dissertation, namely Chapters 5 (toxicity of NNRTI-based cART) and 6 (effectiveness of efavirenz-based cART compared with nevirapine). In this respect, two main pharmacodynamic effects evaluated were the risks for central nervous system toxicity and immunological failure in relation to efavirenz exposure and SNPs in the CYP2B6 composite and CYP2A6.

To begin with, Tables 8.4A and 8.4B show the risks for sub-therapeutic, therapeutic and supra-therapeutic exposure according to the selected SNPs in CYP2B6, CYP2A6, CAR and UGT2B7. It is evident that mutant SNPs in the CYP2B6 and CYP2A6 significantly increases the risk of exposure to supra-therapeutic levels of efavirenz and conversely lower risk for sub-therapeutic exposure in this cross-section of randomly selected plasma samples from patients on efavirenz-based cART. Therefore analysis was restricted to these 3 alleles.

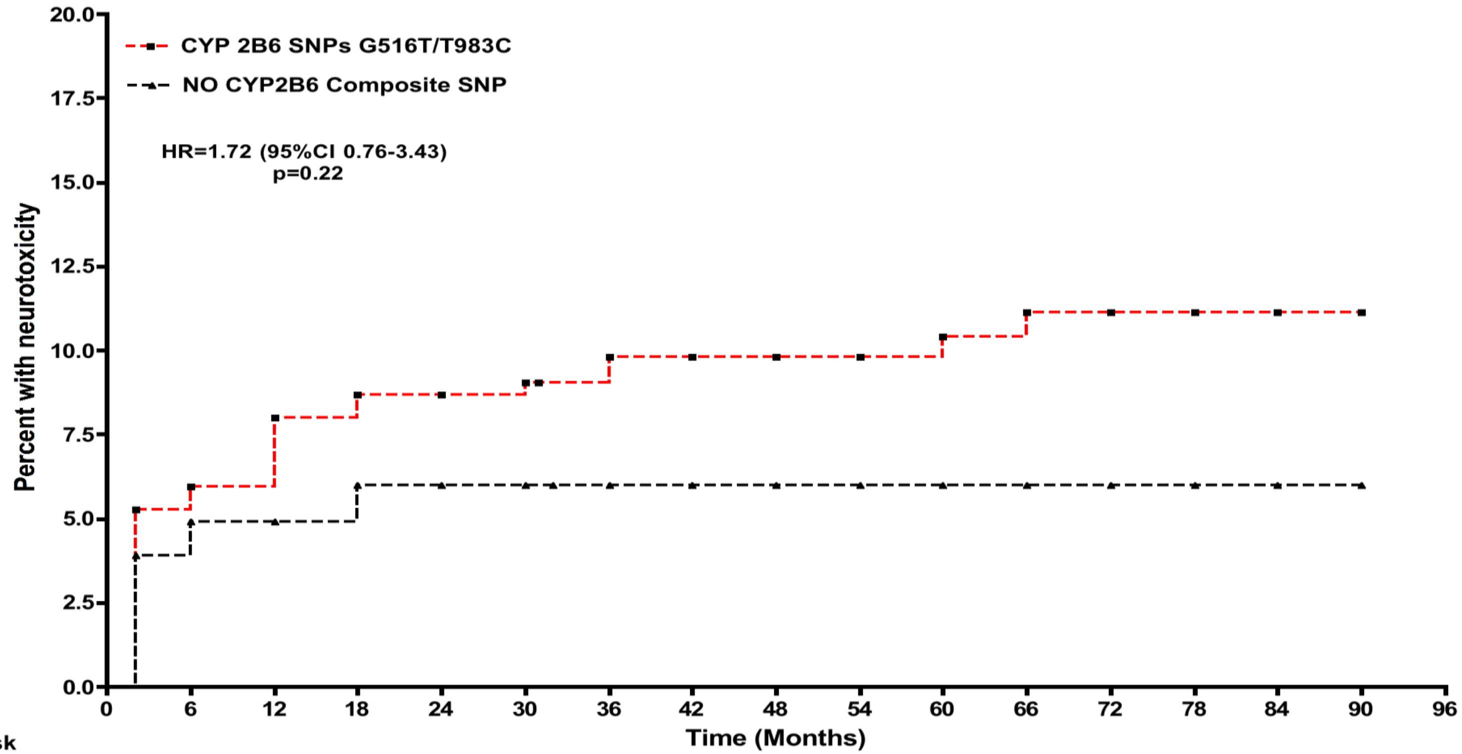
Table 8.4A. Chi-squared analysis for trend between selected SNPs and three clinically relevant therapeutic levels of plasma efavirenz concentrations among a random sample of patients on efavirenz-based cART.

		Clinically relevant plasma efavirenz exposure				
SNP (no. of patients)	Variant status	Sub-therapeutic exposure EFV concentration <1000ng/ml	Therapeutic exposure EFV concentration 1000-4000ng/ml	Supra-therapeutic exposure EFV concentration >4000ng/ml	Chi-square test, df	p-value
CYP2B6 983T>C n=493	wild type	219	200	30	77.73, 2	<0.0001
	mutant type	5	18	21		
CYP2B6 516G>T n= 496	wild type	70	53	5	10.86, 2	0.0044
	mutant type	152	171	45		
CYP2A6*9B n=475	wild type	208	201	38	16.96, 2	0.0002
	mutant type	8	11	9		
UGT2B7*1A n= 494	wild type	161	161	40	0.83, 2	0.66
	mutant type	62	59	11		
UGT2B7*2 n=488	wild type	119	127	28	0.72, 2	0.70
	mutant type	100	91	23		
CAR C>T rs2307424 n=484	wild type	182	189	44	1.16, 2	0.57
	mutant type	35	28	6		

Table 8.4B. The relative risk and odd's ratios of sub-therapeutic and supra-therapeutic exposure to efavirenz according to variants of 6 selected SNPs involved in the metabolism of efavirenz.

SNP (no. of patients)	RR and OR (95% CI) of sub-therapeutic exposure to efavirenz	Fisher's exact test p-value	RR and OR (95% CI) of supra-therapeutic concentrations of efavirenz	Fisher's exact test p-value
CYP2B6 983T>C n=493	0.23 (0.10 – 0.53) 0.13 (0.05 – 0.35)	<0.0001	7.14 (4.49 – 11.36) 12.75(6.34 – 25.63)	<0.0001
CYP2B6 516G>T n= 496	0.76 (0.62 – 0.92) 0.58 (0.39 – 0.87)	0.0099	3.13 (1.27 – 7.72) 3.43 (1.33 – 8.84)	0.0059
CYP2A6*9B n=475	0.61 (0.34 – 1.11) 0.46 (0.20 – 1.07)	0.0783	3.78 (2.04 – 7.02) 5.10 (2.16 – 12.05)	0.0007
UGT2B7*1A n= 494	1.06 (0.85 – 1.31) 1.11 (0.74 – 1.65)	0.6829	0.75 (0.40 – 1.43) 0.73 (0.36 – 1.47)	0.5037
UGT2B7*2 n=488	1.08 (0.88 – 1.31) 1.14 (0.80 – 1.63)	0.5209	1.05 (0.62 – 1.77) 1.06 (0.59 – 1.90)	0.8821
CAR C>T rs2307424 n=484	1.16 (0.89 – 1.50) 1.32 (0.79 – 2.20)	0.2985	0.82 (0.36 – 1.85) 0.80 (0.33 – 1.96)	0.8308

Risk of CNS toxicity according to CYP2B6 composite and CYP2A6 SNPs: 407 patients with data on CYP2B6 composite SNPs and 298 patients with data on efavirenz plasma concentrations were included in this sub-analysis. 37 (9.1%) patients with genomic and 28 (9.4%) with plasma efavirenz data had documented CNS toxicity. Compared with patients without any SNPs in the CYP2B6 composite of G516T/T983C those with SNPs had a non-significant trend towards a higher risk for developing CNS toxicity as shown in Figure 8.5 with a hazards ratio of 1.72 (95%CI of 0.76 to 3.43), p=0.21. At 2 months where most of CNS events occurred, cumulative estimate of events in CYP2B6 mutants compared with wild types was 5.3% (95% CI of 2.8% to 7.8%) vs 3.9% (0.2% to 7.7%). Among this cohort only one event led to efavirenz discontinuation in a patient with heterozygosity for G516T, UGT2B7_802, UGT2B7_735 and wild type for T983C, CAR and CYP2A6*9B but undetectable plasma efavirenz concentration. No significant trends were observed for CYP2A6*9B or plasma efavirenz concentrations from random samples and risk for development of CNS toxicity (not shown).

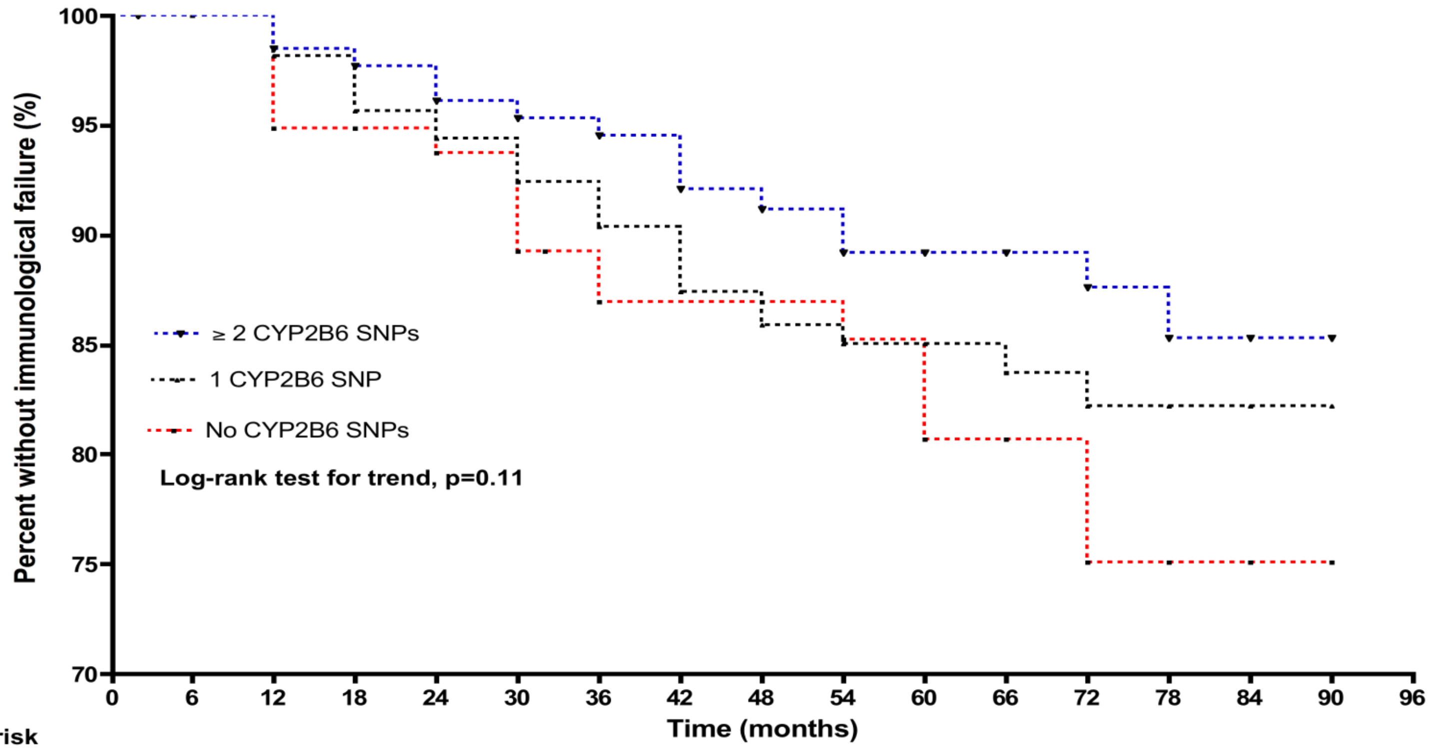


Number at risk

CYP2B6 mutants	304	279	275	258	245	214	147	109	49	17
No CYP2B6 mutants	103	96	91	88	82	77	41	32	17	6

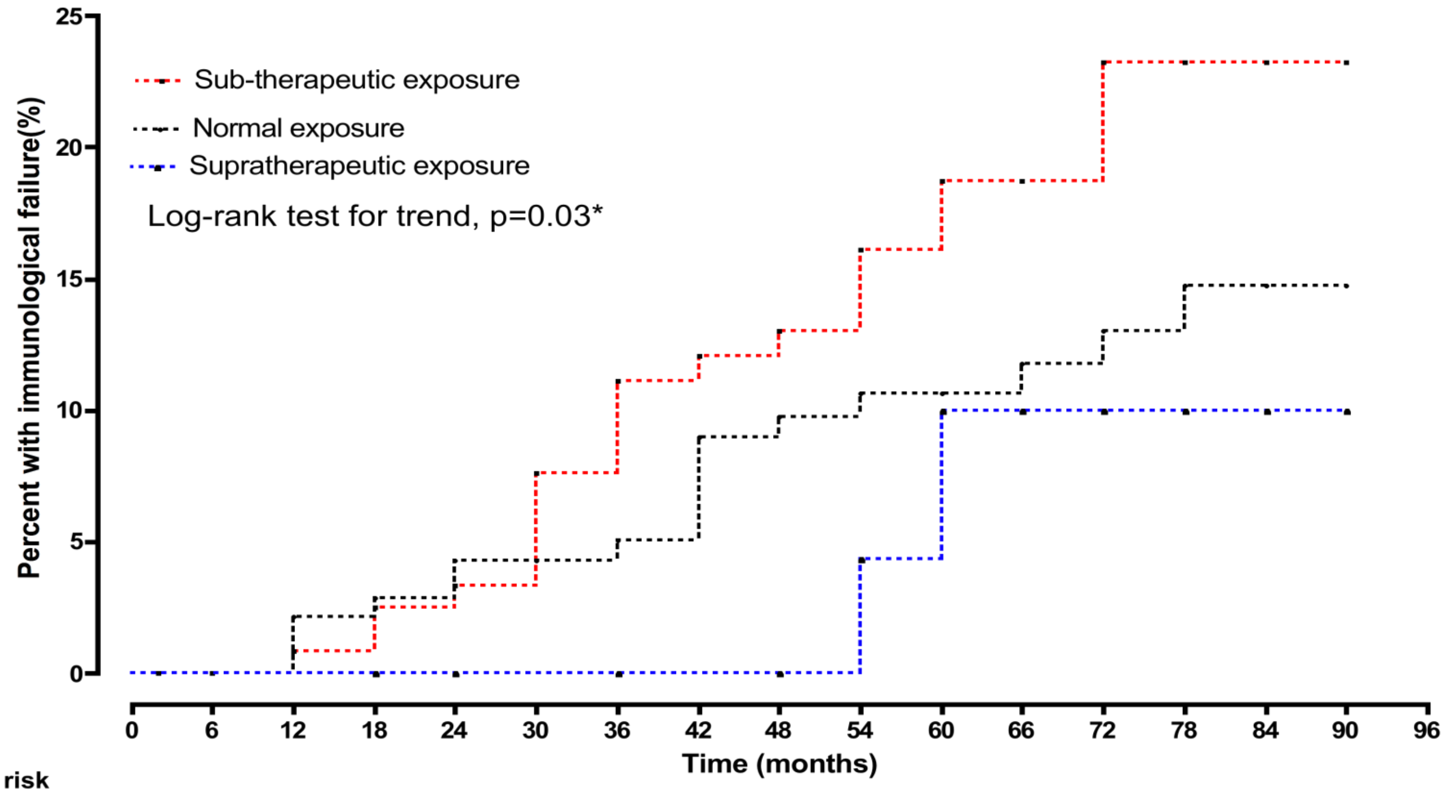
Figure 8.5. Kaplan-Meier risk analysis for reported CNS toxicity due to efavirenz according to SNPs in the composite CYP2B6 G516T/T983C.

b. Risk of immunological failure according to efavirenz exposure and CYP2B6 SNPs: 407 patients with data on CYP2B6 composite SNPs and 298 patients with data on efavirenz plasma concentrations were included in this sub-analysis. 56 patients with genomic data and 42 patients with pharmacokinetic data experienced immunological failure during follow-up on efavirenz-based cART. The median time to immunological failure was 66 months (IQR, of 48 to 78 months). There was a trend towards decreasing risk of immunological failure with increasing number of SNPs in the composite of G516T/T983C as shown in Figure 8.6, although conventional statistical significance level was not reached, p=0.11. Also the hazards ratio for immunological failure from mutant SNPs of CARrs2307424 was 1.42 (95% CI of 0.77-2.63), p=0.26 and that for SNPs of CYP2A6*9B was 1.02 (95%CI of 0.37 to 2.82), p=0.97. Figure 8.7 shows that among a random sample of patients with efavirenz measurements (n=298) in this cohort, there was a significant trend of lower efavirenz exposure predisposing patients towards a higher risk of immunological failure over the long-term and vice versa for supra-therapeutic exposure, log-rank test for trend, p=0.03.



Number at risk									
CYP2B6 no SNPs	103	97	89	77	72	37	29	13	3
CYP2B6 1SNP	170	162	149	134	113	73	55	25	9
CYP2B6 ≥2SNPs	134	131	125	120	104	71	56	23	6

Figure 8.6. Kaplan-Meier risk of immunological failure on efavirenz according to number of SNPs in a composite of CYPB26 G516T and T983C in randomly selected patient samples.



Number at risk									
Sub-therapeutic	125	122	115	105	91	65	54	27	4
Normal	142	140	134	123	116	88	71	31	9
Supra-therapeutic	31	31	30	29	27	17	14	4	1

Figure 8.7. Kaplan-Meier risk of immunological failure on efavirenz according to exposure of efavirenz in randomly selected patient samples.

8.4 Discussion

This study has assessed the frequencies of selected polymorphisms in three enzymes involved in the phase I and II metabolism of efavirenz - CYP2B6, CYP2A6 and UGT2B7-and one nuclear factor (CAR) which modulates induction of CYP2B6 expression, amongst HIV infected Ghanaian patients. In this study population of 800 patients out of which between 85% and 88% of selected SNPs were successfully genotyped, the frequencies of mutant alleles in CYP2B6 516G>T, *983T>C; CYP2A6*9B, UGT2B7 *1A and *2 and CAR C>Trs2307424 were 0.48, 0.04, 0.03, 0.15, 0.23 and 0.07 respectively. 521 patients were on efavirenz with an inter-individual coefficient of variance of 113% corroborating the well-established wide variance in the steady state plasma efavirenz concentrations[122, 561]. SNPs in the CYP2B6 G516T/T983C composite alleles were significant predictors of the efavirenz exposure explaining 32% and 38% of mid-dose efavirenz variance respectively. The impact of mutant SNPs in CYP2A6 becomes pronounced in the presence of loss-of-functions mutations in the CYP2B6 SNPs. Finally, in posthoc retrospective analysis there was a significant trend in the risk for long-term immunological failure on efavirenz-based cART according to plasma efavirenz exposure which was mirrored closely by the number of CYP2B6 SNPs.

Cytochrome P450 2B6 is the major enzyme isoform for the hydroxylation of efavirenz to the major metabolite 8-OH, 8,14-OH efavirenz and the minor 7-OH efavirenz. There are over 100 SNPs described so far with numerous complex haplotypes and different frequencies in different populations[562]. However evidence from several studies show that models inculcating the composite of SNPs in the 516G>T/983T>C predicts efavirenz pharmacokinetics best[335, 340]. In the present study involving only patients with black ethnicity, the frequency of the poor CYP2B6*516G>T metaboliser TT frequency of 25% is comparable with the 23% found among a South African cohort[563], 20% amongst an African-American cohort[276] and 19% in another Ghanaian cohort[282]. Similarly the allelic frequency of CYP2B6 983T>C variants was 91% for homozygous wild type TT and 9% for heterozygous mutants CT which is similar to that observed in the Ghanaian cohort[283]. Only one patient was a homozygous mutant CC for the 983T>C SNP. Thus the minor allelic frequency of 0.04 in the 983T>C SNP in our study closely agrees with that found by Kwara et al[283] among Ghanaians and the 0.045 among West Africans reported by Melhotra et al[35].

In-vitro evidence suggests that efavirenz is one of the selective agents for human CAR mediated CYP2B6 induction[318]. When efavirenz binds to CAR in the cytosol of hepatocytes, this triggers a heterodimer association between CAR and RXR (9-cis retinoic acid receptor), which migrates and binds to response elements in the promoter region of the CYP2B6 gene thus inducing its expression. Until recently, no in-vivo studies had been conducted to assess the contribution of CAR C>Trs2307424 receptor variants on efavirenz steady state exposure particularly among Africans. In a German cohort where 25.5% (n= 373) were of black ethnicity, the variant allele of CAR C>T rs2307424 was present at a frequency of 0.15 among blacks compared with 0.33 among whites[564]. Thus the minor allele frequency in the population of 0.07 in the present study shows that this is a fairly common SNP and certainly further studies are needed to estimate its frequency in other settings. The minor allelic frequency of the CYP2A6*9B gene of 0.03 is comparable with the 0.05 found in another study[283]. Hydroxylated metabolites of efavirenz are subsequently glucuronidated to form an N-glucuronide of hydroxy-efavirenz with the recently identified UGT2B7 playing a predominant role[278].

Kwara et al. reported UGT2B7*1A and *2 minor allelic frequency of 0.26 and 0.46[283] among Ghanaians while 0.15 and 0.23 respectively was found in the present study.

As expected polymorphisms in CYP2B6 516G>T and 983T>C individually (Figures 8.3A and 8.3B) and in combination (Figure 8.4A) were associated with exposure to higher efavirenz mid-dose concentrations as has been noted in several other studies across several ethnicities[209, 335, 340, 565]. On univariate analysis, it was found that variant allele carriers (AC/AA) of the CYP2A6*9B had a 2-fold higher concentration of efavirenz compared to homozygous wild type CC, p=0.0064. A 2-way ANOVA testing for possible gene-gene interaction between the two CYP2B6 SNPs and CYP2A6*9B indicated an absence of significant interaction between the individual 2B6 SNPs and 2A6*9B (Figures 8.4B and 8.4C). Indeed 2A6*9B mutant variants independently had higher levels of plasma efavirenz after adjusting for CYP2B6 516G>T, an effect which was significant among heterozygotes for 516G>T with 2A6*9B mutants compared with 2A6*9B wild types as shown in Figure 8.4B. However this 2A6*9B effect was not observed on adjusting for 983T>C (Figure 8.4C) perhaps highlighting the dominant impact of the 983T>C null allele and the possible reason why 2A6*9B was not a significant independent variable in the multivariate model (Table 8.3). These findings are generally in agreement with those of previous reports [281, 282].

This study is the first to examine the impact of variants of the nuclear factor CAR on efavirenz exposure among Ghanaians and shows overall (Figure 8.3F) the rs2307424 variant of CAR did not significantly influence efavirenz exposure in our study population. The precise mechanistic role of this mutant of CAR remains to be elucidated: looking at Figure 8.4E it is tempting to speculate that it appears to probably exert its effect by enhancing the induction of CYP2B6 in the absence of SNPs in the CYP2B6 G516T/T983C composite allele with a trend towards lower exposure to efavirenz. Kwara et al. were the first to report on the effect of UGT2B7 variants on plasma efavirenz exposure and noted that while carriers of *1A variant had higher efavirenz concentrations than wild type carriers, variant *2 carriers were exposed to lower concentrations of efavirenz compared with wild type[283]. We could not confirm their findings in our study population. Overall, given the large numbers in the present study compared to theirs and the large inter-individual variability in efavirenz exposure, it is likely that our data is better placed to capture the effect of UGT2B7 SNPs if such effects were present. Therefore more studies are required to resolve the impact of UGT2B7 variants on efavirenz exposure in other populations.

Forty-six percent of our participants had sub-therapeutic levels of mid-dose plasma efavirenz concentrations. The finding that nearly half of our participants had sub-therapeutic concentrations of efavirenz is worrying, because of the high risk of selecting resistant strains of HIV. Indeed a recent survey in Johannesburg showed that the frequency of the K103N mutant strains was 25% among patients established on cART[566]. Certainly in programmatic settings where viral loads are not routinely used to monitor treatment efficacy, where therapy options are constrained, where therapy is initiated with non-nucleoside based cART with an inherently low genetic barrier to resistance and where adherence to therapy is assessed by pill counts and patient reporting, this finding should be used by clinicians and peer educators to emphasise and re-enforce the message of adherence to cART. Direct evidence in support of this worrying trend is based on the finding that the risk of immunological failure (Figure

8.7) was associated with plasma efavirenz exposure with the highest risk identified among patients with sub-therapeutic exposure.

It is certainly plausible that the majority of these participants with sub-therapeutic exposure could be non-adherent to their medications. Secondly, there is also the possibility that samples used for the analysis in this chapter most of which were collected in 2006 may have undergone some degradation under storage conditions over time. In support of this, the median (range) plasma concentration of efavirenz in the efavirenz / artesunate pharmacokinetic study (collected in 2010) in chapter seven of this thesis concentration was 2413 (312.9 – 13,060ng/ml) compared with 1087 (110.0 – 12,146.0 ng/ml) for the samples in this chapter. Thirdly, the potential also exists for certain SNPs inducing the so-called "gain-of function" or ultra-rapid metaboliser phenotypic CYP2B6 expression leading to accelerated clearance of efavirenz could partly explain this high proportion of patients with low mid-dose range efavirenz concentration. For instance, a novel haplotype 2B6*22 which combines 4 promoter mutations -1848C>A, -801G>T, -750T>C and -82T>C has been identified among Caucasians DNA samples and demonstrated an enhanced transcriptional activation up to 9-fold compared with the reference CYP2B6 promoter[567]. Furthermore, Leger et al, identified among a Haitian HIV infected cohort who initiated cART with AZT/3TC/EFV and where intake of these medications were observed/supervised that there was a distinct SNP rs36118214 in CYP2B6 which was associated with reduced efavirenz plasma exposure[568]. The minor allele frequency of rs36118214 is thought to be as high as 0.42 among Sub-Saharan Africans[569]. The impact of rs36118214 was not investigated in the present study, but these findings suggest that some patients may be exposed to sub-therapeutic efavirenz concentrations on the basis of their genetic profile.

This could also account for the higher virologic failure rates on the predominantly efavirenz-containing regimens in the AIDS Clinical Trial Group protocol A5095 among blacks compared to whites after adjusting for self-reported non-adherence[580]. In this study we show that the highest risk group for immunological failure were the wild types for both G561T/T983C composite alleles of CYP2B6 followed by those with mutant alleles (Figure 8.6) buttressing the point that genetic profiling may impact treatment outcomes significantly.

Above a steady-state plasma efavirenz concentration of 4ug/l, the risk of central nervous system toxicity increases significantly especially within the first few weeks at initiation of efavirenz-based cART but with continual use patients often develop tolerance to these side effects. In the present study 10% of participants had supra-therapeutic concentrations of efavirenz. The median (range) duration on therapy at pharmacokinetic sampling was 12 months (2 months to 24 months). At this median time point it is expected that majority of patients who were within the supra-therapeutic range would have developed tolerance for CNS toxicity although a formal assessment was not conducted at the time of collecting blood samples in the present study. The potential long- term implications of such sustained exposure to supra-therapeutic levels remains to be determined. However in a retrospective analysis of 407 patients with genomic data, a non-significant trend towards a higher risk for CNS toxicity was observed among mutant carriers of SNPs in the CYP2B6 G516T/T983C genotypes (Figure 8.5) compared with wild type. However only one patient discontinued therapy from efavirenz related toxicity in this subset retrospective analysis precluding any further analysis on associations between genotypic, pharmacokinetic data and risk to treatment-limiting toxicity from efavirenz. As shown in Tables 8.4A and 8.4B, the relative risk of exposure to supra-therapeutic concentrations of efavirenz was significant for mutant allele carriers of the CYP2B6 983T>C, CYP2B6 516G>T and CYP2A6*9B with RRs of 7.14, 3.13 and 3.78 respectively but not the UGT2B7 or the CAR variants. Conversely, the relative risks of sub-therapeutic exposure was significantly lower for mutant carriers of *983T>C and *516G>T but not *9B. It is noteworthy that Ribaudo et al. has reported that one of the pharmacodynamic effects of the composite 516/983 genotypes among black patients is decreased virological failure rate[571]. Alternatively patients with variants of this composite polymorphism are at increased risk of selecting mutant virus on stopping cART because of the prolonged half-life of efavirenz.

The findings in this study show that as a strategy for optimising antiretroviral therapy in Sub-Saharan Africa, therapeutic drug monitoring with dosing of according to genotypic profile may be useful should the technology become accessible in the future. Certainly in this population with a high prevalence of loss-of-function mutations in the metabolic axis of efavirenz, we may be able to achieve effective therapeutic levels with lower doses of efavirenz than is currently recommended for carefully selected patients. Gatananga and colleagues[572] were able to show by using a reduced dose of either 400mg or 200mg of efavirenz for patients with CYP2B6 516G>T SNP, that there was an improvement in CNS related toxicity without a loss of virological suppression. However in countries like Ghana where the goal of adequate access to antiretroviral therapy for all HIV-infected patients remains to be achieved, the added expense of pharmacogenomic genotyping and TDM may seem unrealistic. Furthermore evidence that genotyping and measurement of EFV plasma concentrations actually improve patient outcome is lacking and also most CNS toxicity from efavirenz resolves with continual usage of the medication.

This study had the following limitations worth noting. Samples for this study were collected from a repository without assessing patients for clinical events such as toxicity, treatment failure and adherence prospectively. Therefore adherence was assessed retrospectively by patient self reports and pill counts which has been shown to be reasonably reliable for predicting treatment response[573,574]. In view of the retrospective nature in which the two pharmacodynamic variables- CNS toxicity and immunological failure- were assessed, any associations herein presented should be interpreted with caution and should await confirmation from prospective studies to generalise the import of these observations. Between 12-15% of participants could not be successfully genotyped because there wasn't sufficient genomic DNA in their serum samples. Ideally whole blood sample provide better yield for whole genomic DNA and this would be considered in future studies. These missing data may have biased observations to an extent.

In conclusion, this study shows that there is a high frequency of loss-of-function polymorphisms in the CYP2B6 G516T/T983C composite alleles among Ghanaian HIV-infected patients predicting high exposure to efavirenz. In the presence of mutant SNPs in the CYP2B6, mutant variants of CYP2A6 assume as important role as the sole alternative route for hydroxylation of efavirenz. SNPs in the UGT2B7 and CAR rs2307424 may be of minor relevance in efavirenz exposure among Ghanaians. Interesting trends were observed between CYP2B6 SNPs, random plasma efavirenz concentrations and the risks of CNS toxicity and immunological failure. These are promising findings highlighting the prospects of predictive links between pharmacogenomics and pharmocodynamics of efavirenz via its pharmacokinetic exposure among HIV-infected patients. When the technology becomes accessible in

Sub-Saharan Africa, it may be possible to dose patients on the basis of the genetics and kinetics of efavirenz to maintain patients within therapeutic range to achieve the fine balance between tolerability and efficacy of efavirenz.

CHAPTER NINE

CONCLUSIONS AND RECOMMENDATIONS

This chapter draws the emergent themes and findings of this investigation together, discusses how the research could have been improved and suggests where future research may fruitfully be undertaken.

Purpose for this study

Since 2004, combination antiretroviral therapy for the long-term management of patients living with HIV/AIDS has been available in Ghana where they are administered predominantly through national programmatic settings in ART clinics. Treatment is initiated using a limited repertoire of first line cART comprising of two nucleoside reverse transcriptase inhibitors of either stavudine or zidovudine with lamivudine together with a non-nucleoside reverse transcriptase inhibitor of either efavirenz or nevirapine. This study was conducted because there is a knowledge gap in the Ghanaian ART programme on the long-term effectiveness of cART. Specifically, this study was designed to compare the long-term clinical and immunological outcomes of an efavirenz-based cART compared with nevirapine-based cART within a busy out-patient HIV clinic in Kumasi, Ghana. This was primarily driven by two main observations, the first being that in many similar settings in Sub-Saharan Africa, the effectiveness of efavirenz has not been compared with nevirapine-based cART over the long-term to assess their durability. Secondly, in spite of the toxicity profile of nevirapine, several ART programmes use a predominantly nevirapine-based cART for first line due primarily to cost considerations. It is however anticipated over the coming years that efavirenz may become increasing accessible to many programmes as its cost decreases and this study therefore assessed the tolerability and risk factors for efavirenz related toxicity from three main perspectives: first, from a retrospective analysis of documented events of central nervous system toxicity, hepatotoxicity and cutaneous reactions on efavirenz compared with nevirapine; second, from a pharmacogenomic perspective by assessing the frequencies of selected single nucleotide polymorphisms in the enzymes that are central to the metabolism of efavirenz namely the cytochrome P450 sub-family 2B6, 2A6 and UGT2B7 as well as CAR which is a constitutively expressed androstane receptor required for the induction of the expression CYP2B6 and their impact on the mid-dose exposure of plasma efavirenz concentrations; and third, from a pharmacokinetic perspective by prospectively assessing the interactions and safety of a commonly administered antimalarial, artesunate, for the treatment of clinically suspected malaria among HIV-infected patients established on efavirenz-based cART compared with a control group whose HIV-sero-status was unknown.

Final discussion of findings

Combination antiretroviral therapy was commenced for 4,039 patients between January 2004 to December 2010 and at closure of data for analysis on 31st December 2011, 68% of patients were still alive and active in the clinic, 24% were lost to follow up and 8% mortality was documented over 11,236.8 person years of follow-up. The overwhelming majority of patients were started on cART at low CD4 counts and with various AIDS-defining illnesses on a predominantly NNRTI-based cART (99%) of either efavirenz (59%) or nevirapine (40%) with a backbone of stavudine plus lamivudine (52%) and zidovudine plus lamivudine (48%). Overall first-line cART was effective in this cohort and was accompanied by robust CD4+ T-cell count recovery, reduction in the incidence of AIDS-defining events and sustained increment in body mass index among those who remained on cART. With only 154 (3.8%) patients switching to second line cART due to immunological failure and the profound state of AIDS-associated morbidities of most patients at initiation, first line cART might be considered successful over the long-term in this cohort on the basis of the evidence available from this analysis. This notwithstanding, the lack of viral load data means that treatment success of first-line ART in this cohort may have been over-estimated. For instance, in a retrospective cohort of the first 237 Ghanaian HIV-infected patients initiating cART in Kumasi with follow up to 3 years, although CD4 responses were robust, 6 out of 40 patients with viral load data had virological failure[575]. Similarly, in the HEPIK cohort (Hepatitis B HIV Coinfection in Kumasi), up to 35% (n=300) of patients on first-line cART had evidence of virological failure (personal communications with Prof. Anna Maria Geretti, University of Liverpool, UK) without documented immunological failure. The poor correlation between virological and immunological responses to cART is well characterised[269-271]. In settings such as ours where CD4 counts are used to monitor treatment outcomes, the effectiveness of second line cART could be severely compromised as patients might fail virologically on first line cART for a prolonged time period, accumulate drug resistance mutations before immunological failure is detected.

Efavirenz-based cART was comparable to nevirapine-based cART overall in a composite outcome measure of treatment failure that comprised deaths, disease progression and all-cause treatment discontinuations due either to toxicity, immunologically/clinically determined treatment failure or patient/physician preference with an adjusted HR and OR of 1.20 (95% CI of 0.97 to 1.49), p=0.10 and 1.09 (95% CI of 0.79 to 1.49), p=0.61 in primary analysis. This combination of outcomes has been termed by some authors as the 'durability' of cART[5, 576]. There was a higher overall risk of treatment discontinuation on nevirapine compared with efavirenz due predominantly to treatment-limiting mucocutaneous drug reactions and severe hepatotoxicity. Indeed two deaths from severe muco-cutaneous reactions were associated with nevirapine. Overall, efavirenz was better tolerated with central nervous system toxicity such as insomnia, headaches and dizziness, which although were present at a documented frequency of 7.6% (n= 2,376) among patients on efavirenz led to only 17% (n=180) treatment discontinuations with resolution of these symptoms in the majority over long-term use.

Mutant single nucleotide polymorphisms in genes controlling the production of enzymes and proteins involved in the hepatic metabolism of efavirenz were commonly found in this Ghanaian population of 800 HIV-infected patients. Genotyping by allelic discrimination using real-time PCR was successful in approximately 85% of samples. As expected SNPs in the composite of CYP2B6 *G516T and *T983C were predominantly associated with higher exposure to plasma efavirenz and predicted 32% and 38% of inter-patient variation in plasma efavirenz exposure respectively. This study has shown that in settings where polymorphisms in the CYP2B6 oxidative pathway for efavirenz are common, the CYP2A6 pathway assumes importance as an alternative oxidative metabolic pathway with mutants in *9B predicting even higher efavirenz exposure in the presence of CYP2B6 mutants. It was retrospectively determined that loss-of-function mutants in the CYP2B6 composite of *G516T and *T983C were associated with a trend towards increased risk for CNS toxicity and reduced risk for long-term immunological failure on efavirenz in a subset analysis. Thus the data presented in this study corroborates the important role of mutant SNPs in CYP2B6 in plasma efavirenz with the potential for predicting clinical outcomes such as toxicity and long-term treatment response such as risk for immunological failure while UGT2B7 and CAR may be less important in this regard.

Patients on efavirenz tolerated co-administered artesunate well with no documented toxicity from either medications and clinical failures of malaria treatment assessed on day 5 after completing antimalarial treatment with artesunate therapy. The mid-dose plasma concentrations of efavirenz did not significantly change during the first 6-hours after the first dose of 200mg of oral artesunate and also on day 5 after 6-hours after the last dose.

In essence the findings from this study suggests that efavirenz is effective clinically and immunologically and is well tolerated among this Ghanaian cohort a significant majority of whom started with advanced HIV disease. Polymorphisms in the enzymes responsible for metabolism of efavirenz are highly prevalent among this Ghanaian cohort and predicted efavirenz therapeutic exposure particularly the CYP2B6 G516T and T983C. The CYP2B6 516/983 composite was the most significant in predicting supra-therapeutic exposure to EFV, trends towards increased risk of CNS toxicity but lower risk of long-term immunological failure. Finally even though potential interactions and safety concerns have been proposed for co-administration of artesunate in patients on efavirenz, this study shows that the two medications can be safely administered and were well-tolerated with no significant changes in the mid-dose plasma concentrations of efavirenz.

Representativeness and generalisability of the conclusions

The study has strengths worth noting. This study was conducted in a resource constrained country within a programme setting with the challenges of large patients numbers, limited variety of antiretroviral therapy options and laboratory monitoring as it pertains to many ART programmes in Sub-Saharan Africa and therefore the findings may be applicable to other such settings. Although it is noted that nevirapine is more frequently used in other settings within SSA, this cohort had nearly 60% of patients starting an efavirenz-based cART, thus provided the opportunity to conduct this study where the effectiveness of efavirenz could be assessed in comparison with nevirapine. The cohort in this study is one of the largest to be reported from a single treatment site in SSA with a median follow up of 30 months (range of 0 to 90 months) hence permitting long-term treatment outcomes of first line cART to be assessed. Again the demographic and clinical/laboratory characteristics of patients initiating cART in this cohort were consistently representative and similar to several other cohorts reported from similar settings. These qualities of the study make the data representative and the conclusions drawn on the long-term effectiveness of cART generalisable to many such ART programmes in SSA.

Again the sample size for the pharmacogenomic study of 800 is among the largest to be conducted in SSA to date and was drawn from a clinic where the patients came from at least 8 out of the 10 regions of Ghana representing a significant ethnic diversity for this analysis. Pharmacogenomic data presented are comparable with those of other studies from Sub-Saharan Africa with a high prevalence of SNPs in the CYP2B6 516/983 composite with higher risk of supra-therapeutic exposure to efavirenz. This study highlights the potentials for use of genotyping to tailor the dosing efavirenz and therapeutic drug monitoring to assess adherence should the technology become available in the future. Although this is not in routine use in Ghana and certainly across most ART programmes in Africa, they may serve as useful adjuncts to enhancing adherence to cART in a setting where options are limited.

The use of artesunate monotherapy although not encouraged is practised in some settings like ours. There has been considerable difficulty in coming out with recommendations for antimalarial use among HIV-infected patients on cART by institutions such as the WHO due to the complex predicted interactions and safety concerns between antimalarials and antiretrovirals. Although results of other trials evaluating these questions have been completed and awaiting publication, this study provides some reassuring data on the safety of artesunate when co-administered with efavirenz.

Limitations of the research design

There are important limitations to this study that are noteworthy. The long-term effectiveness of cART was assessed retrospectively in a cohort with important biases such as information and indication biases that are characteristic of such observational studies. Thus although adjusted analysis has been presented throughout this dissertation, there still could be unmeasured biases for which reason, the data presented on effectiveness should be interpreted with these limitations in mind. Data on HIV typing is conspicuously missing from this analysis because this testing was inconsistently performed routinely for patients. This is important because HIV-2 is inherently resistant to NNRTIs and only 40 patients were started on a PI-based cART due to either HIV-2 mono- or HIV1/2 dual-infections out of an estimated 200 or so patients given the prevalence of 4% in the Ghanaian population[577]. It therefore likely that a proportion of patients with HIV-2 infection were started inadvertently on an NNRTI-based cART. Furthermore, although 410 patients had immunological failure by WHO definition on independent analysis, only 154 patients were switched on account of immunologically determined treatment failure by clinicians treating these patients. Finally reported incidences of AIDS-defining events, IRIS, non-AIDS-defining events, toxicity were based on documented events in patient folders and were classified by the author together with the local supervisor. Because these classifications were conducted based on clinical descriptions in patient folders, there is the possibility of mis-classifications and diagnostic overlaps.

The pharmacogenomic study was conducted in retrospectively stored samples from a sample repository hence adherence at the time of sampling was not assessed, thus the correlations between efavirenz plasma concentrations, genotypic data and the predicted associations between efavirenz exposure and SNPs with central nervous system toxicity and risk of immunologic failure should be interpreted within the context of this limitation. Again, this study cannot conclude on the impact of efavirenz on the pharmacokinetics of artesunate or its metabolite Dihydroartemisinin due to degradation of these compounds which is suspected to have occurred during the preparation of these samples for assaying drug levels of the anti-malarial.

Defence of methods

Data for assessing long-term effectiveness was collected from patient folders by the author due to incompleteness of data that was stored in clinic database at the Public Health unit. Given the considerable number of folders that had to be examined, it was felt at the preparation stages to randomly select a sample folders and examine them but it was thought that the best way to have an accurate overview of treatment effect was to document the incidence rates of events such as toxicity, immunological failures, AIDS defining events and non-AIDS-defining events was by assessing all the patients who started therapy within the first 7 years of the ART programme in Kumasi. The advantage of this approach was that it allowed for sub-set analysis to be performed without losing statistical power to draw inferences and conclusions. Again, setting up this database will have potential future use for further follow-up prospective analysis of treatment effectiveness.

In assessing treatment outcomes, loss-to-follow up was a major confounder in analysis since it represented an unmeasured treatment outcome. As much as 24% of patients were lost to follow up in this cohort. Therefore in analysing the composite outcome measure of treatment failure on either efavirenz-based or nevirapine-based cART, analyses were performed with right-sided censoring by considering missing=censored when patient had not experienced any of the co-primary outcome measures or missing= treatment failure (as sensitivity analysis). The former approach assumes that missing patients might not have experienced the composite outcome measure of interest while under follow up (best case scenario) while the latter assumes that missing patients may have experienced any of the components the composite treatment failure as a reason for their 'missingness' (worst case scenario). The premise of the latter assumption was corroborated by direct evidence from a survey of contacts of patients who were lost to follow up which confirmed among respondents that those lost to follow up had died or were still not assessable because of inactivation of telephone numbers. Either way, the conclusions on the effectiveness of efavirenz or nevirapine-based cART were comparable using both assumptions. Methodologically, the potential bias that could have been introduced by censoring may have been approached by using an inverse probability weighting modelling which could be employed to account for missing data when subjects with missing data cannot be included in the primary analysis. Alternatively, a competing risk analysis where loss-to-follow up is considered as a competing risk for death could have been undertaken. But these statistical methods were beyond the authors' scope.

Future research and recommendations for improvement of clinical care.

This cohort had a high pre-treatment attrition from the ART programme with only 38% of those registering for ART between 2004 and 2010 starting therapy. Although no analysis was performed on this subject matter, it is suspected from personal observations from patients folders that most of these patients may have died. From my experience at the clinic, most of these patients present with several AIDS-defining conditions for which treatment for opportunistic infections are initiated while preparations to initiate cART are made by taking patients through adherence counselling. This period could last between 3 to 8 weeks and constitutes a potential delay in initiating cART. Given the success of cART that was observed among those who initiated therapy even with advanced disease, early initiation of cART could serve to reduce the pre-cART attrition. Future research should be directed towards identifying the characteristic differences if any between those who initiate cART and those who did not intiate cART and to formulate strategies for ameloriating this high pre-treatment attrition from the ART programme in Kumasi. Although there are no simple solutions, the author proposes the following: first, admitting the very sick HIV-infected patients who present for cART for supervised treatment of opportunistic infections and where necessary initiation of ART in order to identify toxicity and progression promptly; second, cART could be initiated while pre-treatment adherence counselling proceeds with education given principally to the adherence monitors of prospective patients; third, home visits by supporting network of allied health personnel of the clinic for those who register for ART to be monitored actively.

It became evident from the analysis, that the use of stavudine was associated with a significantly higher risk of death, loss to follow-up and mitochondrial toxicity. Thus the strategy to replace stavudine with tenofovir as part of the first-line cART is possibly a step in the right direction given the high prevalence of hepatitis B co-infection among this cohort for which tenofovir has potent antiviral activity against. However this approach calls for careful and frequent monitoring of renal function using urine analysis and serum biochemistry to identify potential nephrotoxicity because of the high prevalence of baseline renal impairment in this population.

There is also the need to assess the long-term virological outcomes of first line cART among Ghanaian HIV-infected patients. This is because there is a low genetic barrier for resistance to NNRTI and although this study shows robust and sustained CD4 increases on cART, viral loads might help clarify treatment success better as discussed earlier. Importantly there is an urgent need to identify the levels and profiles of drug resistance in patients failing virologically to assess potential treatment options for second line therapy. Previous studies in other African cohorts have identified high frequencies of M184V and Thymidine analogue mutations (TAMS) among patients with virological failure implying that patients may start second line with full resistance to 3TC (and FTC) and partial or full resistance to other NRTIs[578]. Because second-line cART in our ART programme is constructed around 2NRTIs plus a PI (boosted lopinavir the only available option), the PI should be very potent, tolerable and should have a high genetic barrier given that the NRTI base may have been compromised. Whether boosted lopinavir fulfils these criteria for second line PI for sub-saharan Africa remains to be determined. However an analysis of outcomes of second line therapy in our cohort in Kumasi (not presented as part of the main thesis) showed a less robust CD4 recovery, high frequency of clinical events (AIDS-defining events, loss to follow up and deaths) on the predominantly ritonavir-boosted lopinavir-based second line cART. Whilst the need for viral load monitoring can not be overemphasised, there is also the need to increase our repertoire of second line medications especially robust, affordable and novel strategies such as boosted-darunavir used on an optimised backbone or as a monotherapy as has been shown in the MONET[579] study as well as other classes of antiretroviral medications as patients stay longer on first line.

Prospective pharmacogenomic studies where viral loads, clinical events such as toxicity could be collected to substantiate these preliminary observations of the associations between efavirenz pharmacogenomics, pharmacokinetics and pharmacodynamics. In the present study no viral loads could be performed as part of the pharmacogenomics study principally because there wasn't sufficient funding and also samples had been stored at -

20^{o}C under conditions were electrical power supply was not always assured thus the integrity of samples stored for viral loads could not be guaranteed. This study could not conclude on the impact of efavirenz on the pharmacokinetics of artesunate and this requires further studies particularly those that would involve artemisinin-combination therapies and the NNRTIs. A prospective evaluation of long-term neuro-psychiatry side effects of efavirenz on the basis of genotypic data would be useful in assessing the impact of polymorphisms in CYP2B6 *G516T and *T983C on the risk of persistent CNS toxicity on efavirenz. Alternatively, a study randomising patients with persisting CNS toxicity after 2-3 months of efavirenz to (‘genotypically- and therapeutic drug monitoring-determined’) reduced doses of EFV compared with ‘standard care’ to assess success in terms of achieving sustained viral load suppression with improved CNS toxicity profile. Such a study would provide useful proof-of-concept data on the potential of treating patients to target on lower doses of efavirenz on the basis of the high frequency of SNPs in the CYP2B6 in our population.

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APPENDIX 1: ETHICAL APPROVALS

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES
COMMITTEE ON HUMAN RESEARCH PUBLICATION AND ETHICS

Our Ref: CHRPE43/09 May 18, 2009

Dr Richard Phillips
Department of Medicine
SMS-KNUST
Dear Sir,

LETTER OF APPROVAL
Protocol Title: ***A Prospective Study of Pharmacokinetic Interaction between Efavirenz and Artemisinin-based Antimalarial Drugs in Ghanaian HIV Infected Patients***

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee has considered the ethical merit of your submission and approved the protocol.

The Committee wishes to state however, that samples and data gathered for the study should be used for study purposes only. It is recommended that permission should be sought from the committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee would expect a periodic report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publications arising from the study.

Many thanks for your application.

Yours faithfully,

Prof. Sir JW Acheampong, MD, FWACP
Chairman

Private Mail Bag, University Post Office, Kumasi, Ghana. Phone: 233-51-60303. Fax 233-51-60302
E-mail: dean.sms@knust.edu.gh website: www.knust.edu.gh

KWAME NKRUMAH UNIVERSITY OF SCIENCE AND TECHNOLOGY
COLLEGE OF HEALTH SCIENCES
SCHOOL OF MEDICAL SCIENCES
COMMITTEE ON HUMAN RESEARCH PUBLICATION AND ETHICS

Our Ref: CHRPE44/09 May 18, 2009

Prof. George Bedu-Addo
Department of Medicine
SMS-KNUST

Dear Sir,

LETTER OF APPROVAL
Protocol Title: ***A Study Investigating the Effect of Polymorphisms of the CYP2B6 Cytochrome and other Genetic Determinants on Efavirenz and Nevirapine Levels in Patients attending the KATH HIV Clinic***

Your submission to the Committee on Human Research, Publications and Ethics on the above named protocol refers.

The Committee has considered the ethical merit of your submission and approved the protocol.

The Committee wishes to state however, that samples and data gathered for the study should be used for study purposes only. It is recommended that permission should be sought from the committee if any amendment to the protocol or use, other than submitted, is made of your research data.

The Committee would expect a periodic report on your study, annually or at close of the project, whichever one comes first. It should also be informed of any publications arising from the study.

Many thanks for your application.

Yours faithfully,

Prof. Sir JW Acheampong, MD, FWACP
Chairman

Private Mail Bag, University Post Office, Kumasi, Ghana. Phone: 233-51-60303. Fax 233-51-60302
E-mail: dean.sms@knust.edu.gh website: www.knust.edu.gh

Appendix 2: Proforma for collection of data on HIV-infected patients who initiated cART in Kumasi.

CODE		AGE		SEX				WHO		Conditions			
Date enrol			Date started			Last visit		HIV type			HBV status		
Town/City		Region		Height		Previous ART		Patient			Contact		
Date	Months follow-up	CD4 count	Weight	HB	AST	ALT	Creatinine	Septrin	ART	Adherence	Toxicity	diagnoses	IF/CF/VF
	0												
	2												
	6												
	12												
	18												
	24												
	30												
	36												
	42												
	48												
	54												
	60												
	66												
	72												
	78												
	84												
	90												
	96												
	102												
TC		TG		LDL-C		HDL-C			FBS		Comments		

Appendix 3: Clinic record forms for registration, initial patient assessment, follow-up clinical care on antiretroviral therapy and laboratory data.

REGISTRATION FORM

Date: ______/______/_______) (dd/mm/yyyy)

Health Facility: ________________________________

PATIENT IDENTIFICATION

Patient's Name:______________________ Registration No:______________

Residential Address:______________________ Region ______________

Postal Address:________________________________

Telephone No: Home:____________ Work____________ Mobile____________

Date of Birth (if known):____________ Age:__________ Gender: ☐ Male ☐ Female

Marital status: ☐ Married ☐ Single ☐ Divorced ☐ Separated ☐ Widow (er) ☐ Cohabiting

Occupation: ____________________ Status: ☐ Full time ☐ Part time ☐ On leave ☐ Unemployed

Education (*Tick one*): Nil ________ Primary_______ JSS_______ MSLC_______ Sec/Tech______ Tertiary______

Religion *(Tick one):* Muslim________ Christian________ Traditional________ None_________ Other_________

Dependent children (Less than 18 yrs)

Age	Sex	Alive or Dead	Status Pos,Neg, Don't Know)	HIV Care (Y/N)	Age	Sex	Alive or Dead	Status (Pos, Neg, Don't Know)	HIV Care (Y/N)
1					4.				
2.					5.				
3.					6.				

EMERGENCY CONTACT

Name:________________________ Relationship:________________

Home Address:________________________________

P. O. Box:________________________ City/Town:________________

Telephone No: Home:____________ Work:____________ Mobile:____________

REFERRAL INFORMATION

☐ Diagnostic HIV testing ☐ Walk-in VCT site ☐ PMTCT program ☐ Old Patient ☐ Transfer In ON OI

☐ Transfer In ON ART ☐ TB ☐ STI ☐ Others

FUNDING

Funding Type: ☐ Patient out of pocket ☐ Medical insurance: Type: ____________ ☐ Special project

☐ Employee sponsored: Name of Employer: ____________________ ☐ Other: ______________

☐ Waiver (date requested:________________)

Date of Death_____/_______/_______(dd/mm/yyyy)

CLINICAL CARE — INITIAL ADULT ASSESSMENT FORM

Date of HIV+ Test: ___/___/___ **(dd/mm/yyyy)** **HIV Type:** HIV I ___ HIV II ___ Both HIV I & HIV II ___

Vital Signs: Height(cm): ___.__ Weight (kg): ___.__ Temp (°C): ___.__ Pulse (bpm): _____ B/P: ___/_____

TB Screening: Yes____ No____ If yes, Result: ☐ Suspect____ ☐ Non Suspect____

If Suspect, TB Diagnosis: Pos ____ Neg ____ Other____

Drug Allergies: ______________________________

Current Medications:

For opportunistic infection prophylaxis:

Cotrimoxazole: Yes ☐ No ☐ Fluconazole: Yes ☐ No ☐ TB Treatment: Yes ☐ No ☐

For other conditions:

Medication Name: ______________________ Medication Name: ______________________

Herbal Preparation ☐

Past ARV experience: Yes ___ No ___ *If Yes,* list drugs and dates

Drug________________________ Duration (taken from what date to what date) ____________________

Drug________________________ Duration (taken from what date to what date) ____________________

Drug________________________ Duration (taken from what date to what date) ____________________

Women Only:

ARV Prophylaxis for PMTCT: Yes___ No___ If yes, date started: ____/____/____ (use dd/mm/yyyy)

Baby treated? Yes ___ No___ If yes, date started: ____/____/____ (use dd/mm/yyyy)

HISTORY AND PHYSICAL EXAM FINDINGS

Presenting Problem (in patient's words):

Symptom Screen

Condition	Current	Past	Never	Other Acute/Chronic Conditions	Yes	NO
Jaundice				Hypertension		
Chronic cough				Asthma		
Difficulty swallowing				Sickle Cell Disease		
Skin rash, itching				Hepatitis		
Chills				Diabetes		
Fever				Heart Disease		
Severe weight loss (>10%)				Malaria		
Vomitting				Others specify		
Sexually transmitted infections						
Persistent Headaches						
Visual Changes						
Pain: ___________ Location						
Abnormal Menses						
Oral thrush						
Others specify						

Family Planning: Using Family Planning? Yes ☐ No ☐ If Yes, Method ______________________

Women only: Pregnant? Yes ☐ No ☐ Exp Delivery Date____/____/____ (dd/mm/yyyy)

1

Habits:

Smoking: Yes____ No____ Ex_____ If Yes: Type___________ # /day or week_________ Duration: #/yrs ________

Alcohol: Yes____ No____ Ex______ If Yes (Check one): Every day______ >Once weekly ____ >Once monthly____

HIV Disclosure And Family Response:

Disclosed____ Not disclosed ____

If disclosed: Disclosed to: Family __ (Supportive__ Not Supportive__) Friend __ (Supportive __ Not Supportive __)

Sources of emotional support: Family____ Friends____ Other____ If Other Specify:______________________

PHYSICAL EXAM

General description of patient presentation:

Physical Findings

General Appearance	Yes	No	Skin	Yes	No
Pallor			Pruritic Papular Dermatitis		
Febrile			Abscesses		
Dehydrated			Kaposi's lesions		
Jaundiced			Herpetic lesions (e.g. Zoster)		
Peripheral edema			Seborrheic dermatitis		
Other findings			Fungal infections		
Lymphatic System			**Gastrointestinal**		
Lymphadenopathy			Hepatomegaly		
Oral			Splenomegaly		
Oral hairy leukoplakia			Tenderness		
Oral thrush			Distension		
Oral lesions			Other findings		
Other findings			**Neurological**	**Abnormal**	**Normal**
Respiratory			Orientation to person, time, place		
Rate: __________	_______	_______	Speech		
Labored breathing			Neck stiffness		
Cyanosis			Blindness one/both eyes		
Wheezing			Hemiplegia/paresis (R or L or both)		
Intercostal recession/ subcostal recession			Numbness of extremities		
Auscultation findings:			Other findings		
Cardiac	**Abnormal**	**Normal**	**Mental Status**	**Yes**	**No**
Heart rate and rhythm			Slow mentation		
Auscultation findings (include murmurs)			Memory loss		
Breasts	**Yes**	**No**	Mood swings		
Lumps, masses			Depression		
Discharge			Anxiety		
Genitalia			Suicidal ideation		
Vaginal/urethral discharge			Seizures		
Genital ulcer, other lesion			**Others**		
Inguinal node enlargement					

TRANSMISSION RISK FACTOR ASSESMENT

Is Patient sexually active?	Yes ☐ No ☐	*If Yes*, continue with questions below
Disclosure to sexual partner?	Yes ☐ No ☐	If no, refer to Adherence Counselor for prevention counseling
Regular condom use?	Yes ☐ No ☐	If no, refer to Adherence Counselor for prevention counseling

WHO CLINICAL STAGE

WHO Stage 1		**WHO Stage 4**	
Asymptomatic HIV infection		Candidiasis (Esophageal, Bronchi, Trachea, Lungs)	
Persistent Generalized Lymphadenopathy (PGL)		Crytococcosis, Extrapulmonary	
WHO Stage 2		Cryptosporidiosos with Diarrhoea (> 1 month)	
Herpes Zoster (within the last 5 years)		Cytomegalovirus Disease	
Minor Mucocutaneous Manifestations		Herpes Simplex (mucocutaneous > 1month; or visceral any duration)	
Recurrent Upper Respiratory Tract Infections		HIV encephalopathy	
Weight Loss < 10% of Body Weight		HIV Wasting Syndrome	
WHO Stage 3		Kaposi's Sarcoma	
Severe Bacterial Infections(i.e., pneumonia)		Lymphoma	
Oral Candidiasis (Thrush)		Atypical Mycobacteriosis, Disseminated	
Unexplained Chronic Diarrhoea (> 1month)		Tuberculosis, Extrapulmonary	
Unexplained Prolonged Fever (intermittent or constant >1 month)		Progressive Multifocal Leukoencephalopathy (PML)	
Oral Hairy Leukoplakia		Mycosis, Disseminated (ie Histoplasmosis, Coccidiodomycosis)	
Tuberculosis, Pulmonary (within previous year)		Pneumocystis Carinii Pneumonia (PCP)	
Weight Loss (> 10% of Body weight)		Salmonella Septicemia, Non-typhoid	
Unexplained Anaemia		Toxoplasmosis, CNS	

☐ **WHO Stage I** ☐ **WHO Stage II** ☐ **WHO Stage III** ☐ **WHO Stage IV**

Diagnostic Findings

☐ Malaria ☐ STI ☐ Diabetes ☐ Hypertension ☐ Pregnancy

Others:

ARV ELIGIBILITY CRITERIA

Clinical and Biological Criteria:	**Yes**	**No**	**Comments**
CD4 below 350			Baseline CD4:_______
WHO Stage III or IV			
Completed intensive phase of TB therapy			
Completed pre-treatment adherence counseling sessions			
Social Criteria:			
Resident of catchment area			
Disclosure to selected other person			

3

1. OI Prophylaxis:

OI Prophylaxis Assessment (Stage II, III, IV): If yes to any of the below conditions, treatment before prophylaxis may be indicated. **Investigate further before starting prophylaxis.**

	Yes	No		Yes	No	
Cough			Jaundice			
Allergy			Pregnant			

1. (a) Cotrimoxazole: Start ___ Continue ___ Discontinue ___ Start at a later date ___
   (b) Fluconazole: Start ___ Continue ___ Discontinue ___ Start at a later date ___
   (c) TB Treatment: Start ___ Continue ___ Discontinue ___ Start at a later date ___ Completed Treatment___

2. **Hospital Admission:** Yes _____ No _____

3. **Order: Baseline labs:** Yes _____ No _____
   If Yes state labs……………………………………………………………………………………………………
   Pregnancy Test: Yes _____ No _____

4. **Treatment for other conditions** Yes _____ No _____
   If Yes:
   Diagnosis: ______________________________ Treatment: ___________________________
   Diagnosis: ______________________________ Treatment: ___________________________
   Diagnosis: ______________________________ Treatment: ___________________________

5. **Recommend ARV Treatment:** Yes ____ No ____ IF YES REFER TO ADHERENCE COUNSELOR
   IF NO State reason: ____

6. **Adherence Counseling:**
   Referred to Adherence Counselor: Yes _____ No _____
   If Yes, Name of Counselor ________________________________

7. **Adherence Sessions:**
   1st Session Date:____/_____/____ (dd/mm/yyyy)
   2nd Session Date:____/_____/____ (dd/mm/yyyy)
   3rd Session Date:____/_____/____ (dd/mm/yyyy)

8. **Next scheduled appointment**: ___/___/___ (dd/mm/yyyy)

9. **Special Comments**

Medical Officer / Physician's Name: ________________________________ **Signature:** __________________

CLINICAL CARE

ADULT FOLLOW - UP VISIT FORM

Date: ______________________ (dd/mm/yyyy) Follow Up Visit Number ________________

Scheduled Visit? Yes______ No______ If yes, did patient come on the date of appt.? Yes _____ No _____

VITAL SIGNS:

Weight (kg):_____.__	**Temp (°C):** _____.____	**Pulse (bpm):**_______	**B/P:** ______/______

Client on ART? Yes______ No _____ *If No, DO NOT COMPLETE ART Sections*

Client on CTX prophylaxis? Yes__ No__ Client on Fluconazole prophylaxis? Yes__ No__ Client on TB Treatment? Yes__ No__

SKIP IF CLIENT IS ON TB TREATMENT

TB Screening: Yes____ **No** _____ **If yes, Result:** ☐ **Suspect**____ ☐ **Non Suspect** ____

If Suspect, TB Diagnosis: Pos ____ **Neg** ____ **Other** ____

PATIENT COMPLAINTS (FOR ALL PATIENTS) TICK all that apply

Patient chief complaints (in patient's own words)

Symptoms		Symptoms		Symptoms	
Cough		Lipodystrophy/Lipoatrophy		Pain (Site:________________________)	
Dyspnea on exertion		Nausea		Headache	
Haemoptysis		Vomitting		Dizziness	
Difficulty in swallowing		Diarrhoea		Insomnia	
Skin rash		Abdominal discomfort		Abnormal dreams	
Fever/chills		R quadrant pain		Anxiety/Depression	
Blood in urine		Myalgia/Arthralgia		Mental changes(cognitive acuity)	
Fatigue		Paresthesia		Others:________________________	

PHYSICAL EXAMS FINDINGS :

General description of patients presentation:

System	Normal	Abnormal	System	Normal	Abnormal
General Appearance			Genitalia		
Lymphatic System			Gastrointestinal/Liver/Spleen		
Oral			Skin		
Respiratory			Neurological		
Cardiac			Mental Status		
Breasts					

Note any abnormal findings

Family Planning: Using Family Planning? Yes ☐ No ☐ If Yes, Method ____________________________

Women only: Pregnant? Yes ☐ No ☐ Exp Delivery Date____/ ____ /____ (dd/mm/yyyy)

TRANSMISSION RISK FACTOR ASSESSMENT

Is Patient sexually active?	Yes ☐	No ☐	*If Yes*, continue with questions below
Disclosure to sexual partner?	Yes ☐	No ☐	If no, refer to Adherence Counselor for prevention counseling
Regular condom use?	Yes ☐	No ☐	If no, refer to Adherence Counselor for prevention counseling

1

Adherence for ARVs and CTX last 3 days before this visit

	0 pill missed	1 - 2 pills missed	3 - 4 pills missed	5 > pills missed
ARVs (self-reported)				
CTX (self-reported)				

Patient's reason for missing doses: (check all that apply. FOR PATIENTS WHO MISSED DOSES ONLY)

Unable to pay		Too many pills		Too busy to take		Did not want to take it	
Felt well		To avoid side effects		Felt sick/ill		Shared pills with others	
Forget to take		Ran out of pills		Felt depressed/anxious		None	

WHO CLINICAL STAGE – FOR PATIENTS NOT ON ART – Skip If patients is On ART

WHO Stage 1		**WHO Stage 4**	
Asymptomatic HIV infection		Candidiasis (Esophageal, Bronchi, Trachea, Lungs)	
Persistent Generalized Lymphadenopathy (PGL)		Crytococcosis, Extrapulmonary	
WHO Stage 2		Cryptosporidiosos with Diarrhoea (> 1 month)	
Herpes Zoster (within the last 5 years)		Cytomegalovirus Disease	
Minor Mucocutaneous Manifestations		Herpes Simplex (mucocutaneous > 1month; or visceral any duration)	
Recurrent Upper Respiratory Tract Infections		HIV encephalopathy	
Weight Loss < 10% of Body Weight		HIV Wasting Syndrome	
WHO Stage 3		Kaposi's Sarcoma	
Severe Bacterial Infections(i.e., pneumonia)		Lymphoma	
Oral Candidiasis (Thrush)		Atypical Mycobacteriosis, Disseminated	
Unexplained Chronic Diarrhoea (> 1month)		Tuberculosis, Extrapulmonary	
Unexplained Prolonged Fever (intermittent or constant >1 month)		Progressive Multifocal Leukoencephalopathy (PML)	
Oral Hairy Leukoplakia		Mycosis, Disseminated (ie Histoplasmosis, Coccidiodomycosis)	
Tuberculosis, Pulmonary (within previous year)		Pneumocystis Carinii Pneumonia (PCP)	
Weight Loss (> 10% of Body weight)		Salmonella Septicemia, Non-typhoid	
Unexplained Anaemia		Toxoplasmosis, CNS	

☐ **WHO Stage I** ☐ **WHO Stage II** ☐ **WHO Stage III** ☐ **WHO Stage IV**

Diagnostic Findings

☐ Malaria ☐ STI ☐ Diabetes ☐ Hypertension ☐ Pregnancy

Others:

ARV ELIGIBILITY CRITERIA – FOR PATIENTS NOT ON ART – Skip If Patient is On ART

Clinical and Biological Criteria:	**Yes**	**No**	**Comments**
CD4 below 350			Baseline CD4:_______
WHO Stage III or IV			
Completed intensive phase of TB therapy			
Completed pre-treatment adherence counseling sessions			
Social Criteria:			
Resident of catchment area			
Disclosure to selected other person			

2

PATIENTS ON ART - Skip if Patient is NOT on ART

ADVERSE CLINICAL EVENTS: Check all that apply

Tuberculosis (Pulmonary)		Weight Loss after starting ART		Cryptococcal Meningitis	
Pneumonia		STI		Malaria	
Oral/Esophageal Candidiasis		Herpes Zoster		Immune Reconstitution Syndrome	
Chronic Diarrhoea		Karposi's Sarcoma		Others: ____________	

ADVERSE DRUG SYMPTOMS: Check all that apply

Anaemia		Hepatotoxicity		Depression	
Rash		Pain/numbness/tingling in extremeties		Specify below any Severe drug reaction	
Diarrhoea > 3days		Blood in Urine			
Pancreatitis		Lipodystrophy			

ART Treatment Failure? Yes ☐ No ☐ Not determined ☐

If yes, select Type of failure: Clinical ☐ Immunological ☐ Virological ☐

PLAN – FOR ALL PATIENTS

1. **OI Prophylaxis**

(a) *Cotrimoxazole:* *Start ___ Continue ___ Discontinue ___ Start at a later date ___*

(b) Fluconazole: Start ___ Continue ___ Discontinue ___ Start at a later date ___

(c) *TB Treatment:* *Start ___ Continue ___ Discontinue ___ Start at a later date ___ Completed Treatment___*

2. **Hospital Admission:** Yes _____ No _____

3. **Order: Labs:** Yes _____ No _____ Pregnancy Test: Yes _____ No _____

4. **Treatment for other conditions** Yes _____ No _____

If Yes:

Diagnosis: ______________________ Treatment: ______________________

Diagnosis: ______________________ Treatment: ______________________

Diagnosis: ______________________ Treatment: ______________________

5. **ARV Status:**

Start ARVs _____

Re-start ARVs ___________

Continue ARVs _____

Change ARVs _____ Reason: Drug Toxicity ___ Treatment Failure ___ TB Diagnosis ___

Stop ARVs _____ Reason: Death ___ Adverse Clinical Status/event ___ Lost to Follow-up ___

Client Stopped ARVs Reason: Drug Side Effects ___ Felt Well ___ Unable to Pay ___

PMTCT Prophylaxis _____

PMTCT Treatment _____

Start ARVs at a Later Date _____

6. Transfer Client to other ART site: Yes _____ No _____

If yes, name of Site ______________________ Date of Transfer ___/_____/____ (dd/mm/yyyy)

TREATMENT PRESCRIBED : (check all that apply)

FIXED DOSE COMBINATIONS			
AZT(Zidovudine) + 3TC (Lamivudine) + NVP (Nevirapine)			
AZT(Zidovudine) + 3TC (Lamivudine) + EFV (Efavirenz)			
d4T (Stavudine) + 3TC (Lamivudine) + NVP (Nevirapine)			
d4T (Stavudine) + 3TC (Lamivudine) + EFV (Efavirenz)			
AZT(Zidovudine) + 3TC(Lamivudine)			
AZT (Zidovudine) 300 mg			
d4T (Stavudine) 30 mg			
3TC (Lamivudine) 150 mg			
NVP (Nevirapine) 200 mg			
EFV (Efavirenz) 600 mg			
EFV (Efavirenz) 800 mg			
NFV (Nelfinavir) 250mg			

ddI (Didanosine) 250mg			
ddI (Didanosine) 400mg			
(Lopinavir/Ritonavir) 400/100mg			
ABC (Abacavir) 300mg			
TDF (Tenofovir) 300 mg			
NFV (Nelfinavir) 250mg			

Has Treatment Regimen been switched? Yes ______ No ______

Other drugs prescribed: 1.____________________ 2. ____________________ 3.____________________

4.____________________ 5.____________________

Referred to Adherence Counselor: Yes _____ No ____

Next appointment scheduled: ____/____/____ (dd/mm/yyyy)

Any other comments

Medical Officer/Physician's Name: ______________________________Signature: ______________________

PATIENT ID: ____________________________

Name of Patient: ____

Sex:______________ Age: _________

HIV Type: a) HIV -1 only b) HIV -2 only c) Both HIV 1 & 2

LAB INVESTIGATIONS

Test / Date	CD4	Viral load	Hb	WBC(total)	Total Lymphocyte Count(TLC)	ESR	INH Dipsticks	Total Bilirubin	Direct Bilirubin	Indirect Bilirubin	SGOT/aspartate Aminotransferase (AST)	SGPT/alanine Aminotransferase (ALT)	Alkaline Phosphatase (ALP)	Serum Albumin	Serum Globulin	Hepatitis B Surface Ag	G6PD

Amylase	Na	K	Urea	Creatinine	FBS/RBS	Urine R/E	Stool R/E	B/F for malaria	Uric acid	Serum Cholesterol	LDL Cholesterol	HDL	Triglycerides	Sputum for AFBs	CXR	CD8	GGT

Appendix 4

Clinical Report Form:
A prospective study of pharmacokinetic interactions between efavirenz and artemisinin-based antimalarial drugs in Ghanaian HIV patients

Information given? Y ☐ N ☐
Consent obtained? Y ☐ N ☐
Did patient fulfil criteria of capacity? Y ☐ N ☐

Clinic no............................

Study no ☐ _ _ _ ☐☐

Inclusion/Exclusion

Inclusion
Age 18+? Y ☐ N ☐
On Efavirenz for > 2 weeks + >90% adherence (only if HIV+ on treatment)? Y ☐ N ☐

Must answer yes to above

Exclusion
Does patient suffer with malabsorption disorder? Y ☐ N ☐
Is the patient pregnant? Y ☐ N ☐
Has patient vomited in last 24 hours? Y ☐ N ☐
Is the patient taking any other tablets known to affect cytochrome P450 or P-glycoprotein? Y ☐ N ☐

Must answer no to above

If the patient is taking Lumefantrine:
a) has the ECG been checked? Y ☐ N ☐ and b) is the QTc < 450ms? Y ☐ N ☐
has the ECG been attached? Y ☐ N ☐

N.B. Must answer <u>yes to all inclusion criteria</u> & <u>no to all exclusion criteria</u> to participate

Demographics/HIV Information

Age........ or Date of Birth............................ Sex M ☐ F ☐
Past Medical history:

Diagnosis	Date diagnosed

Does the patient suffer with epilepsy? Y ☐ N ☐

Date of HIV Diagnosis.................. Latest CD4 Count........... ....date............

Current HIV Medications:

Drug	Dose	Frequency	Date started

Previous HIV Medications:

Drug	Dose	Frequency	Date stopped

Clinical Report Form: A prospective study of pharmacokinetic interactions between
efavirenz and artemisinin-based antimalarial drugs in Ghanaian HIV patients

Clinic no..................
Study no _ ___ ___ ___

Other current medications:

Drug	Dose	Frequency	Date started

Traditional medicines taken in last 4 weeks:
....

Adherence

Is patient taking Efavirenz? Y ☐ N ☐
Did the patient take Efavirenz last night? Y ☐ N ☐
Has the patient been taking Efavirenz for >2 weeks? Y ☐ N ☐
How many times has the patient missed their Efavirenz tablet in the last 4 weeks?
0 ☐ 1-2 ☐ 3-4 ☐ 5-6 ☐ 7-8 ☐ 9-10 ☐ >10 ☐
How many times has the patient missed their Efavirenz tablet in the last 1 week?
0 ☐ 1-2 ☐ 3-4 ☐ 5-6 ☐ 7-8 ☐ 9-10 ☐ >10 ☐

Day 1 (day of recruitment) date........................

Does the patient currently suffer from:			Details
Chest pain?	Y ☐	N ☐	....
Palpitations?	Y ☐	N ☐	....
Dizzyness?	Y ☐	N ☐	....
Blackouts?	Y ☐	N ☐	....
Shortness of breath?	Y ☐	N ☐	....
Fever?	Y ☐	N ☐	....
Headache?	Y ☐	N ☐	....
Weakness of arms or legs?	Y ☐	N ☐	....
Difficulty walking?	Y ☐	N ☐	....
Difficulty sleeping?	Y ☐	N ☐	....
Nightmares or vivid dreams?	Y ☐	N ☐	....
Difficulty in concentrating on tasks?	Y ☐	N ☐	....
Suicidal thoughts?	Y ☐	N ☐	....
Visual disturbance?	Y ☐	N ☐	....
Speech disturbance?	Y ☐	N ☐	....
Hearing problems?	Y ☐	N ☐	....
Fits/faints/funny turns/ involuntary movements?	Y ☐	N ☐	....
Numbness/pins and needles?	Y ☐	N ☐	....
Tremor?	Y ☐	N ☐	....
Diarrhoea?	Y ☐	N ☐	....
Nausea or Vomiting?	Y ☐	N ☐	....
Rectal bleeding/melaena?	Y ☐	N ☐	....
Jaundice (yellowing of skin)?	Y ☐	N ☐	....
Itching?	Y ☐	N ☐	....
Rashes?	Y ☐	N ☐	....

Any other symptoms? Please specify...

Clinical Report Form: A prospective study of pharmacokinetic interactions between
efavirenz and artemisinin-based antimalarial drugs in Ghanaian HIV patients

Clinic no..................
Study no _ ___ ___ ___

Physical Examination at baseline (day 1)

Observations: BP........./............ Temperature.........ºC Pulse............/bpm RR...............

System	Normal	Abnormal	Details
Cardiovascular	☐	☐	
Respiratory	☐	☐	
Abdominal	☐	☐	

Neurological

Does the patient have any of the following:

	Yes	No	Details
Ataxia?	☐	☐	
Dysarthria (slurred speech)?	☐	☐	
Hearing loss?	☐	☐	
Weakness?	☐	☐	

Any other neurological deficit?...

If the patient complains of any neurological symptoms, specifically do they have abnormalities of:

	Normal	Abnormal	Details
Cranial nerves?	☐	☐	
Reflexes?	☐	☐	
Power of arms/legs?	☐	☐	
	Yes	No	
Nystagmus?	☐	☐	

Once the above has <u>all</u> been completed, please ensure patient has bloods taken for

- Urea/Electrolytes + Liver Function Tests + Full Blood Count + Malaria Film ☐

- Pharmacokinetic samples (at times 1 hour, (2 hours), 4 hours and 6 hours, to be sent to UK) ☐

Clinical Report Form: A prospective study of pharmacokinetic interactions between
efavirenz and artemisinin-based antimalarial drugs in Ghanaian HIV patients

Clinic no..................
Study no _ ___ ___ ___

If the patient complains of any neurological symptoms, specifically do they have abnormalities of:

	Normal	Abnormal	Details
Cranial nerves?	☐	☐	
Reflexes?	☐	☐	
Power of arms/legs?	☐	☐	
	Yes	No	
Nystagmus?	☐	☐	

Adherence

How many Efavirenz tablets have you missed since your last clinic appointment 4 days ago?

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

How many Artesunate tablets have you missed since your last clinic appointment 4 days ago?

0 ☐ 1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

Please ensure patient has bloods taken for

- **Urea/Electrolytes + Liver Function Tests + Full Blood Count + Malaria Film** ☐
- **Pharmacokinetic samples – 6 hours post dose (to be sent to UK)** ☐